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The First-Feed Study: Milk intake, energy balance and growth in infants exclusively breast-fed to 6 months of age

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Thesis submitted in fulfilment of the requirement for the degree of

Doctor of Philosophy (Ph.D.) by Research to

College of Medical, Veterinary & Life Sciences

School of Medicine

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Scotland



Under supervision of

Professor John J Reilly

Submitted September 2012

Author's Declaration

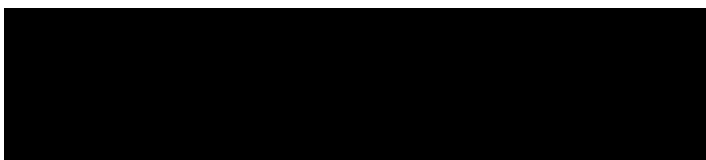
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Susan Bjerregaard Nielsen

Supervisor's declaration

"I certify that the work reported in this thesis has been performed by Susan Bjerregaard Nielsen and that during the period of study she has fulfilled the conditions of the ordinances and regulations governing the Degree of Doctor of Philosophy"



Professor John J Reilly

Summary

The World Health Organization (WHO) recommends exclusive breast-feeding until 6 months of age¹, where exclusive breast-feeding is defined as giving human breast milk only with no other foods or fluids². This recommendation has since been adopted by many countries. A systematic review of studies in exclusively breast-fed infants by Reilly and colleagues³ found a mean milk intake at 6 months of age that seemed too low to cover infant energy requirements. However, the evidence was relatively scarce, only from cross-sectional studies and based on the method of test-weighing, which has been criticised for under-estimating milk intake. Furthermore, longitudinal studies indicated no marked increase in milk intake over time, but these studies did not include measurements at 6 months of age³. Reilly and Wells proposed the hypothesis that for exclusive breast-feeding to adequately cover infant energy requirements to 6 months of age, either 1) infants had to be unusually small, or 2) breast milk energy content had to be unusually high, or 3) milk intake had to be unusually high⁴. The Reilly-Wells hypothesis was backed up by evidence of a world-wide low prevalence of exclusive breast-feeding to 6 months⁵, and by studies consistently reporting a maternally perceived insufficient milk supply as a major reason for mothers to cease exclusive breast-feeding and introduce either formula supplementation or complementary foods⁶.

Based on the Reilly-Wells hypothesis, the research question for the First-Feed study was: To explore how exclusive breast-feeding to 6 months of age is achievable - mainly from an energy balance point of view. The First-Feed study tested the hypothesis that successful exclusive breast-feeding to 6 months of age would include 1) infants that were small and/or growing slowly, 2) milk intakes and/or milk energy content that were higher than literature values and increasing over time, 3) infant energy requirements that were lower than reference values, and/or 4) infant feeding practices that were strained by very frequent and/or very time consuming breast-feeds. The study was designed as the first longitudinal observational study to use an isotopic method to measure milk intake and energy balance in exclusively breast-fed infants to 6 months of age, and it evaluated parts of the methodology employed in the study, in order to appreciate the results in light of the methodological strengths and limitations.

The First-Feed study found that infants were overall of normal size and growing well relative to WHO Child Growth Standards. Metabolisable milk intakes were significantly higher than the values obtained by Reilly and colleagues³ at both 3½ and 6 months of age, and increased significantly over time. Infant energy requirements, determined as metabolisable energy intake, was significantly higher than references for mean energy requirements⁷ at 3½ months of age, while it was appropriate at 6 months of age. Breast-feeding practices showed no change over time in feeding frequency, but a significant decrease in time spent on breast-feeds.

The First-Feed study had several limitations. Firstly, due to the inclusion criteria of exclusive breast-feeding, the participants were characterised as an affluent and well-supported sample of mother-infant pairs, who were highly motivated to breast-feed. Therefore, the generalisability of the present study to other populations should be accepted with caution. Secondly, the anthropometric measurements were prone to imprecision, as is often the case in field studies. Thirdly, the imprecision of the dose-to-infant procedure for administration of doubly-labelled water considerably reduced the precision of the doubly-labelled water method. This, in addition to the biological variation, increased the variation in some outcome variables. However, the First-Feed study is unique as it is the first to use a more objective method to measure milk intake in a longitudinal design, and on a sample of infants with a very high success rate of exclusive breast-feeding to 6 months of age.

The WHO changed the recommendation on exclusive breast-feeding from 4 - 6 months to 6 months (exactly) in 2001¹. Since then, many resources have been invested in breast-feeding promotion, but rates of initiation, duration and exclusivity is only slowly improving⁸. The present study supports that exclusive breast-feeding can adequately cover infant energy requirements to 6 months of age - even without undue strain on breast-feeding practices and even in mothers where initial breast-feeding problems were very common. However, the present study found a wide variation in both infant size, milk intake and energy requirements. It therefore begs the question if a recommendation based on one age-point (6 months exactly) is appropriate given the vast biological variation in variables that are important for the adequacy of exclusive breast-feeding, or if the recommendation should be adapted to include developmental milestones (e.g. oral motor skills) indicative of readiness for complementary foods.

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The present thesis would never have become a reality were it not for my supervisor, Professor John J Reilly, who has been a very understanding and invaluable mentor all the way; ready to patiently guide me when I was in doubt, and leave me to work, when I was confident I could do so. Also invaluable for the present study was our collaborator from University College London, Professor Jonathan C.K. Wells, who made the isotope analyses possible, and also offered freely of his great knowledge and expertise. The hypothesis tested in the present thesis was generated by Professors Reilly and Wells, prior to the present study. Together with them, and also with Dr. Mary Fewtrell from University College London, ideas were discussed and the design of the present study gradually took shape. I named it the First-Feed study: “For Infant Research STudies: breast-Feeding Exclusively for an Extended Duration”. I achieved ethical approval, recruited participants and collected data by conducting the study as a field study. For this part, I feel the greatest gratitude to the mums and their babies, who invited me into their homes and willingly offered their time and commitment for free. Many have since been in touch with anecdotes and pictures, and several of the babies who participated in the First-Feed study now have younger siblings. For the mass spectrometry analysis of urine samples, and for generating the isotope data, I owe great thanks to James Grinham, and also to Eva Miriam Craig, who helped with data-entry of breast-feeding questionnaires and diaries. Also many thanks to Dr. David Young, who provided advise on statistical analysis, and to my advisor, Professor Charlotte Wright, who regularly checked up on me and my progress. More generally, I feel a great sense of gratitude to the international community of breast-feeding experts, where I have received such great encouragement and inspiration at conferences and meetings; Professors Lawrence Weaver, Kay Dewey, Dan Sellen, Peter Hartman and Miriam Labbok just to name a few. A research grant for the First-Feed study was provided by the Scottish Government Chief Scientist Office, and I received financial support from Blegdalen’s Erhvervs og Uddannelsesfond (Blegdalen’s Business and Educational Fund), and subsequently a studentship from Yorkhill Children’s Foundation, for which I am very grateful. Finally, I would like to acknowledge my husband and two children, who have been very supportive and have tolerated me every day throughout this Ph.D.

Preface

A measurement is just a way of reducing an observation to a simple value. Many of such values can then be summarised and tested against expected values, distributions or trends. Measurements will always depend on the method used to obtain them, and the circumstance under which they are obtained. Furthermore, observations can be much more than the value resulting from a measurement. When conducting the First-Feed study, I made many observations. Reflections on such observations helped me gain a strong anecdotal experience of what exclusive breast-feeding in Scotland is like. It also allowed me to reflect on more philosophical aspects of science, make grateful acknowledgements of what I've learned and also humbly face the daunting mountains of what I don't yet know or understand. Such reflections are part of a continuous process that creates a scientist. The present thesis merely reflects my status in this process, when it was submitted, but the process itself is a never-ending journey. It is a fantastic privilege to be given the opportunity to humbly report the First-Feed study in such detailed form, and this is my story...

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List of abbreviations

ALSPAC	Avon Longitudinal Study of Parents and Children
BBD	Baby Behaviour Diary
BFQ	Breast-feeding questionnaire
BMI	Body Mass Index
BMR	Basal Metabolism Rate
CF	Complementary breast-fed (breast milk plus complementary foods)
CHSP-PS	Child Health Systems Programme Pre-school system
DARLING	Davis Area Research on Lactation, Infant Nutrition and Growth
DONALD	Dortmund Nutritional and Anthropometric Longitudinally Designed
DXA	Dual-energy X-ray Absorptiometry
DLW	Doubly-labelled water
EBF	Exclusive breast-feeding or exclusively breast-fed (used in figures)
E_{growth}	Energy stored in growth
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology And Nutrition
FAO	Food and Agriculture Organization of the United Nations
FFM	Fat Free Mass
First-Feed	For Infant Research Studies: breast-Feeding Exclusively for an Extended Duration
FM	Fat Mass
FQ	Food Quotient
HIV	Human Immunodeficiency Virus
IFPS	Infant Feeding Practices Study
IGF-1	Insulin-like Growth Factor 1
IMD	Index of Multiple Deprivation (England)
IQ	Intelligence Quotient
IWL	Insensible Water Loss
IRMS	Isotope Ratio Mass Spectrometry
ISD	Information Services Division (Scotland)
LCPUFA	Long-Chain Poly-Unsaturated Fatty Acids
MGRS	Multicentre Growth Reference Study
NCT	National Child birth Trust
NHS	National Health Service
RQ	Respiratory Quotient
SACN	Scientific Advisory Committee on Nutrition

±SD	Standard Deviation
SIMD	Scottish Index of Multiple Deprivation
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
TBW	Total Body Water
TEE	Total Energy Expenditure
TEI	(metabolisable) Total Energy Intake
TEM	Technical Error of the Measurement
UK	United Kingdom
UNICEF	United Nations Children's Fund
UNU	United Nation's University
WHO	World Health Organization

The present thesis is concerned with how exclusive breast-feeding for the first 6 months of life is achievable. It is mainly concerned with energy balance, and does not consider other aspects of exclusive breast-feeding, e.g. nutrient adequacy of exclusive breast-feeding or the effect of exclusive breast-feeding on infant psychosocial development, although a few aspects of breast-feeding practices are included.

In 2001, the World Health Organization (WHO) stated its new global recommendations of exclusive breast-feeding for the first 6 months of life and continued breast-feeding for 2 years and beyond¹. This recommendation has provoked quite a debate. Firstly, a simplified global recommendation may fail to recognise the variety of situations in which it is to be implemented⁹. Secondly, the evidence base for the recommendation is largely derived from observational studies, which do not have the highest evidential value. Thirdly, the acclaimed benefits of breast-feeding may vary with provenance and in clinical importance. Finally, the prevalence of exclusive breast-feeding is low in many countries^{5,8,10} and thus the WHO recommendation seems unrealistic to achieve for any meaningful proportion of many populations.

An often reported reason for ceasing exclusive breast-feeding, is a maternally perceived insufficient milk supply, although this phenomenon has never been confirmed in terms of actual evidence of inadequate milk supply^{6,11,12}. The evidence of the volume of milk intake during exclusive breast-feeding to 6 months is scarce - partly because it is a relatively uncommon practice and partly because methods of measuring milk intake can be imprecise and prone to underreporting^{13,14}.

This thesis is a report of the First-Feed study; a longitudinal observational study using the doubly-labelled water (DLW) method to measure milk intake and energy balance in infants exclusively breast-fed to 6 months of age. The study also included measurements of anthropometry to ascertain growth, and questionnaires and diaries to explore breast-feeding practices. With these data, the present thesis will test the hypothesis proposed by Reilly and Wells^{3,4} and explore how exclusive breast-feeding is achievable for the first 6 months of life.

Chapter 1 describes “the setting” of breast-feeding, including the definitions and terminology used in the literature, recommendations on breast-feeding, possible risks and benefits of breast-feeding as well as breast-feeding prevalence and factors influencing this. It also reviews the evidence of milk intake in relation to infant energy requirements.

Chapter 2 describes the methodology used in the First-Feed study, and provides an account of how the First-Feed study was conducted. Chapters 3 to 7 are results of participant characteristics and infant growth, evaluation of dose-to-infant administration of DLW, lactation performance, infant energy balance, and breast-feeding practices. Chapter 8 is a summarising discussion of the findings of the First-Feed study with implications for breast-feeding promotion and future research.

From the First-Feed study, one paper was published on the aspect of dose-to-infant administration of DLW¹⁵, and one paper was published on the adequacy of milk intake during exclusive breast-feeding¹⁶. Another paper was concerned with infant energy requirements and prediction equations for these¹⁷. Finally, the First-Feed study was presented at various conferences, some of which have resulted in abstract publications. The details of these appear from the list of publications in the author’s bibliography (Appendix C).

1.1 Background on breast-feeding

Breast-feeding is a hot topic in the fields of public/infant health and research, and the literature published is vast and fast expanding. Historically, breast-feeding as a behaviour has never been particularly popular¹⁸, in spite of the fact that it has ensured the survival of mammal species for many millions of years¹⁹. More recently, public health initiatives to promote breast-feeding and enforce changes in maternal behaviours often meet great resistance and intervention studies in the United Kingdom (UK) have had limited success²⁰.

The WHO is a primary stakeholder for global public health, and works to influence breast-feeding practices on a global scale, by collecting evidence, and publishing consensus documents on definitions, recommendations and strategies for member countries to implement. Therefore, within the topics of breast-feeding, infant growth and infant health, the WHO has a prominent voice and will be referred to frequently in the present thesis.

The following sections describe the various definitions and terminology of breast-feeding that are to be found in the literature. It also states the current recommendations and their evidence base, and summarises the main risks and benefits of breast-feeding. It also provides an overview of the most recent rates (prevalence) of breast-feeding, and describes the strongest determinants of breast-feeding initiation and duration.

1.1.1 Definitions and terminology in breast-feeding

Previous lack of consensus on definitions

In studies related to infant and child health, breast-feeding is an important exposure variable, because it is considered likely to affect outcomes such as metabolism, growth, development, morbidity and mortality². However, the literature reveals a lack of consistency in the breast-feeding terminology used in studies investigating these effects of breast-feeding, and this reduces comparability of studies and could lead to misinterpretation of data²¹. This could transpire into reviews, systematic reviews and meta-analyses, where a variety of definitions are included^{22,23}, which then increase variability in outcome measures and make conclusions less clear. Conversely, studies may be excluded due to inadequate definitions of breast-feeding, whereby potentially relevant research may be excluded from the evidence base.

A lack of consensus on breast-feeding definitions and terminology also makes it difficult to assess which extent breast-feeding recommendations are adhered to¹⁰. Therefore, having consensus on definitions and terminology would help countries nationally in their monitoring of breast-feeding prevalence relative to national breast-feeding targets, and also internationally when evaluating and comparing the effect of intervention studies that aim to promote breast-feeding^{10,24}, or when evaluating the effect of breast-feeding itself on health-related outcomes. Breast-feeding should be measured both in terms of duration and in terms of “dose” of exposure, i.e. degree of breast-feeding. However, it is impractical to measure breast milk intake as well as accounting for any complementary foods on a daily basis. Therefore definitions are used, which work as indicators of breast-feeding exposure². Generally, the currently working definitions of breast-feeding relates to the type of nutrient-giving food ingested by the infant, rather than the mode by which the infant receives the food.

However, it can be discussed whether the mode of receiving breast milk also has an impact on infant health and development. The act of breast-feeding has been described as a complex bidirectional biological dialogue between mother and infant where many less well investigated processes are thought to happen, which may affect infant development²⁵.

Earlier suggestions of breast-feeding definitions

There seems to be a historical development in definitions of breast-feeding in concordance with the increasing awareness of its effects on infant metabolism, infant development and short and long term health^{2,26}. In 1990, a schema and framework for definitions of breast-feeding and breast-feeding practices was suggested²¹. In this schema, a distinction was made between *exclusive breast-feeding* - as giving nothing else, but breast milk - and *almost exclusive breast-feeding*, where small amounts of supplements, water, juice or ritualistic feeds could be consumed. Together, these categories were termed *full breast-feeding*. The term *partial breast-feeding* was subdivided into three categories of degrees of breast-feeding and the term *token* was proposed to describe breast-feeding that is very occasional in nature with limited nutritional impact. An additional schema for recording breast-feeding at a given point in time was proposed with the opportunity to describe variables of breast-feeding practices such as frequency, duration and intervals between breast-feeds²¹. Unfortunately, this well-defined schema was never widely implemented.

In 1991, the WHO held a meeting with other international stakeholders (national agencies and international charities) as a first step towards reaching consensus on indicators for assessing breast-feeding practice²⁴. Taking into account the schema described above, the report of the meeting further clarified definitions of breast-feeding, how and when it should be measured, and provided a clear description of the recommendations at the time, which were based on the Innocenti Declaration²⁷ (section 1.1.2). This work continued and ultimately resulted in a consensus update on definitions in breast-feeding as well as indicators to use for measuring breast-feeding incidence and prevalence, which was published by WHO in 2008². These definitions will be used in the present thesis with only one adaptation as described below.

Current definition of exclusive breast-feeding

The definition of *exclusive breast-feeding* is where an infant receives only breast milk and no other liquids or solids, with the exception of oral rehydration solutions, drops or syrups consisting of vitamins, mineral supplements or medicines². This is exclusive breast-feeding as defined by WHO², which will be used throughout the present thesis.

Definitions of non-exclusive breast-feeding

Predominant breast-feeding is defined as where an infant receives breast milk primarily, but supplemented with certain liquids, like juices or ritual fluids in addition to the exception above². The term *almost exclusive breast-feeding*²¹ has also been used to describe this feeding practice, as well as *full breast-feeding*, which was previously used by the WHO and others to describe the two terms of exclusive and predominant breast-feeding together^{10,24}. Merging such feeding practices somewhat disguises the actual degree of breast-feeding, but a study found that the volume of milk intake is not significantly lower during predominant breast-feeding compared to exclusive breast-feeding²⁸. However, supplementation itself may still have an impact on other health-related outcomes, like infections^{29,30}.

Partial breast-feeding has been used as the term for “mixed feeding with breast milk and other sources of energy and nutrients”^{21,31}. Whilst partial breast-feeding is not a term currently used by WHO², it is often used in the literature and will be used in the present thesis when referring to this literature. Some studies have used the term *mixed feeding* as indicating infants fed a mixture of breast milk and formula milk, whilst other studies suggests this as exclusive breast-feeding because the diet is entirely milk based as opposed to complementary feeding, where other food sources, than milk, are consumed³².

The terms *any breast-feeding* or *ever breast-fed*²⁴ or *all breast-feeding*¹⁰ have been used in the literature about the prevalence of breast-feeding that included exclusive, predominant and partial breast-feeding, where breast-feeding might be supplemented with formula and/or complementary foods. In the present thesis, this is termed any breast-feeding.

Definition of complementary feeding

Complementary feeding is the term used by the WHO when infants receive breast milk and any other foods or liquids, including non-human milk and formula milk². Therefore, *complementary foods* comprise “any nutrient-containing foods or liquids other than breast milk”³¹, including formula and human milk substitutes. This term therefore includes all degrees of partial breast-feeding. In the First-Feed study, infants that had been introduced to complementary foods, and were given any amount of complementary foods in addition to breast milk, were termed *complementary breast-fed* infants. This term deviates from the WHO definitions, but it was deemed helpful in the present thesis in order to emphasise that the feeding practice described was one of breast-feeding with a (small) supplement of complementary foods.

The European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition has commented on this definition finding it unhelpful and potentially confusing to include infant formula and human milk substitutes as complementary foods in respect to the situations where exclusive breast-feeding was never established and the infant is supplemented³³.

Generally, the term *solids* or *weaning foods* have often been used interchangeably with the term *complementary foods* in the literature. In the present thesis, only the term *complementary foods* will be used, and the term *solids* is avoided because complementary foods includes solids, semi-solids and liquids when it also includes energy containing drinks and formula milk, according to current WHO definitions². The terms *weaning* and *weaning foods* are avoided in the present thesis since these words indicate a partial or complete cessation and replacement of breast milk when in fact breast milk is meant to be continued with a supplement of complementary foods³¹.

Initiation, duration and exclusivity

The *initiation* of breast-feeding, is breast-feeding as recorded or reported to have taken place within the first week to ten days after birth, and the rate of initiation of breast-feeding is also referred to as the *incidence* of breast-feeding. The *duration* of breast-feeding refers to the infant age at which breast-feeding was ceased, while the term *prevalence of breast-feeding* is used generally to describe the rate of breast-feeding after initiation.

Initiation, duration and prevalence can be described for both exclusive breast-feeding and any breast-feeding. Finally, the term *exclusivity* has been used as a general term, where for instance increasing exclusivity means increasing the incidence and/or the duration of exclusive breast-feeding.

1.1.2 Breast-feeding recommendations

The Innocenti Declaration

In the summer of 1990, the WHO and other co-sponsors met in Florence, Italy, to discuss a global initiative for the promotion of breast-feeding in the 1990's²⁷. From this meeting a declaration was produced, which recognised breast-feeding as the ideal nutrition for infants as well as stating some of the benefits of breast-feeding, and that these benefits were increased with increased exclusivity during the first 6 months of life.

They proposed as a global goal for optimal infant and maternal health that all infants should be fed exclusively on breast milk for the first 4 - 6 months of life. The Innocenti Declaration has been the basis for many governmental policies and targets regarding breast-feeding, and still is, in many countries. Along with the Innocenti Declaration, the WHO, United Nations Children's Fund (UNICEF) and co-sponsors launched the Baby Friendly Hospital Initiative, which comprised of ten simple steps of practices for maternity wards to adopt in order to support the initiation of breast-feeding³⁴. Several studies have found that implementation of the Baby Friendly Hospital Initiative improves the incidence of any and exclusive breast-feeding^{35,36}, also in Scotland³⁷, but a survey found that even where initiation rates increase, an increase in breast-feeding duration does not necessarily follow³⁸.

The World Health Organization's recommendations

In May 2001, the WHO held the 54th World Health Assembly with the topic of "Infant and Young Child Nutrition"¹. The background for this assembly was an acknowledgement that malnutrition of infants and young children remains a severe global public health problem, which has detrimental effects on both immediate and long-term health. The WHO wanted a sound scientific basis for policies to improve growth monitoring as well as infant feeding practices.

As a result of this assembly, the member states of the WHO were urged to promote and support exclusive breast-feeding for 6 months as a global health recommendation and continued breast-feeding for up to 2 years or beyond¹. The recommendation was followed by the WHO “Global Strategy for Infant and Young Child Feeding”, which contained details on how to protect, promote and support exclusive breast-feeding to the recommended duration³⁹.

The recommendation was the conclusion of an expert consultation report, commissioned by the WHO, on optimal duration of exclusive breast-feeding⁴⁰. The objective of the expert consultation was to review existing evidence on the optimal duration of exclusive breast-feeding. It was mainly based on a Cochrane review on studies of the effect of exclusive breast-feeding for 3 - 4 months versus 6 months on infant growth and development⁴¹ and a review on the energy and nutrient adequacy of exclusive breast-feeding for 6 months⁴².

The Cochrane review

For the Cochrane review, the study criteria were controlled clinical trials and observational studies, where each study had their own control group, i.e. studies comparing results with population statistics or other literature, were excluded⁴¹. The infants had to be born at term with no restrictions on birth weight. Breast-feeding had to be specifically defined as exclusive breast-feeding for ≥ 3 , but < 7 months (i.e. introduction to complementary foods between 3 and 6 months) for one group, and another group with exclusive breast-feeding for ≥ 6 months. However, some of studies included in the review had infants that were predominantly breast-fed in the group of exclusively breast-fed. The evidence revealed 2 trials and 17 observational studies that varied in both quality and provenance.

The main finding was no adverse effects on growth, development, infant or maternal health of exclusive breast-feeding to 6 months of age. However, the data were insufficient to exclude several potential risks, including growth faltering and certain micronutrient deficiencies, e.g. a potential risk of iron-deficiency in susceptible infants. Based on these results, the authors supported a recommendation of exclusive breast-feeding to 6 months, and this brought the WHO recommendation in line with UNICEF who had recommended exclusive breast-feeding for “around 6 months” since 1993⁴³. The Cochrane review was updated in 2009⁴⁴ (section 1.1.3).

Review on adequacy of exclusive breast-feeding to 6 months

A review was conducted by Butte and colleagues on the adequacy of energy and protein intake as well as intakes of three vitamins and three minerals during exclusive breast-feeding to 6 months of age⁴². Adequacy was defined in terms of functional outcomes including growth, immune response, neurodevelopment and morbidity. The review concluded that energy intake during exclusive breast-feeding appeared to be adequate to cover infant energy requirements for 6 months, but it was acknowledged that the current evidence had many shortfalls, including a lack of human milk intake studies of 6 months exclusive breast-feeding, especially from developing countries⁴².

Systematic review on complementary feeding

Another systematic review was concerned with the effect of age at introduction of complementary feeding on infant health²². For the studies reviewed, infant formula was not included as a complementary food, so infants that were only fed milk (breast or formula) were grouped together. Thirty-three papers describing 25 observational studies met the inclusion criteria of information on age at introduction of complementary foods and specific information on outcome variables. The review applied nine methodological criteria relevant for observational studies to assess study quality.

None of the 33 papers fulfilled all criteria, and the overall heterogeneity of studies meant that data extraction for a meta-analysis was precluded. Thirteen studies were found to be supportive of the recommendation of exclusive breast-feeding for 6 months, whilst 13 studies supported the recommendation of exclusive breast-feeding for 4 - 6 months, and 7 studies were inconclusive. The conclusion of the review was that the evidence was insufficient to support a change in the recommendations from the 4 - 6 months exclusive breast-feeding to 6 months exclusive breast-feeding, and it emphasised the need for global recommendations to be flexible enough to accommodate certain population sub-groups that may benefit from earlier introduction to complementary foods than the majority²².

Recommendations by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition

ESPGHAN have recently commented on the WHO recommendation and have also reviewed the evidence³² - finding that there is no advanced benefit of exclusive breast-feeding for 6 months versus 4 - 6 months³³. ESPGHAN is more lenient in their recommendation as they describe exclusive breast-feeding for 6 months as a desirable goal, and that complementary feeding other than infant formula and follow-on formula, should not be introduced before 17 weeks (4 months), and no later than 26 weeks (6 months) of age³³.

Recommendation from the UK Government

The UK Department of Health recommended in 1994 that “the majority of infants should not be given solid foods before the age of 4 months, and a mixed diet should be offered by the age of 6 months”⁴⁵, which could be understood as allowing infant formula during the first 4 months of life. The English Department of Health officially adopted the WHO recommendation in May 2003⁴⁶.

Recommendation from the Scottish Government

In Scotland, many health boards have adopted the WHO recommendation and subsequently revised their infant feeding guidelines to both health care professionals and to parents. However, it was not until November 2008 that the Scottish Government officially adopted the WHO recommendation⁴⁷.

1.1.3 Risks and benefits of breast-feeding

The risks and benefits of breast-feeding have been investigated intensely, and the literature on this topic is vast. It is beyond the scope of the present thesis to give an exhaustive account of this literature, but here is an overview of the more well-investigated claims with references to further study.

The quality of evidence

Randomised controlled trials are given superior weight when looking at the quality of evidence, and they are considered the gold standard of research designs^{48,49}. Preferably they should be “double-blind” so that neither participant nor investigator knows which randomisation group the participant is in, but this is not possible for obvious reasons when the exposure is breast-feeding.

Randomised controlled trials have a high internal validity; i.e. the ability to determine causality is beyond that of other study designs, as randomisation reduces the risk of bias and the influence of confounding. However, randomising the exposure of breast-feeding is considered unethical for term-born healthy infants given the current evidence base in support of breast-feeding to improve health. Instead, randomised trials are either in preterm infants⁵⁰, or they include breast-feeding promotion or support as the intervention⁵¹. For the latter, the actual exposure of breast-feeding is not controlled, and data consists of groups with some breast-feeding versus groups with more breast-feeding. A few exceptions to this are the Honduras Trials^{52,53} and a recent study from Iceland⁵⁴, where mothers willing to breast-feed exclusively to 6 months were randomised at 4 months to either continue exclusive breast-feeding, or to introduce complementary foods as controlled by the study design.

Observational studies often suffer from confounding, where factors that are influencing the mothers to breast-feed are also influencing other maternal behaviours that in turn affect health outcomes⁵⁵. Even with careful adjustment for all known confounders, residual confounding still remains a concern. Furthermore, the links between breast-feeding and health outcomes are only associations, and therefore no firm conclusion can be made regarding causality⁵⁶.

Some studies have revealed evidence of a dose-response effect⁵⁷⁻⁵⁹, which may lend some support to the effect being due to breast-feeding rather than the result of confounding.

Other studies raise the concern for reverse causation. For instance, one study found lower air flows (indicative of asthma) in adolescents of asthmatic mothers who had been breast-fed for >4 months compared to those who had been breast-fed for <2 months⁶⁰. However, it is more likely that early indicators of asthma themselves caused the mothers to breast-feed for longer⁶¹.

When looking at the risk of developing a disease as the outcome variable in studies of protective effects as a benefit of breast-feeding, there is also the risk of case selection bias, where the “diagnosis” of a case is influenced by prejudices or expectations⁶². For instance, loose stools may be less likely to be diagnosed as diarrhoea in a breast-fed infant.

Within the evidence from observational studies, a major limitation is the inconsistent and inadequate use of definitions of breast-feeding. In prospective cohort studies, there are issues with drop-outs, and the risk that the reasons for drop-out are associated with health outcomes⁵⁶. In long-term epidemiological studies, breast-feeding, or its cessation, is often retrospectively recorded with a socially patterned recall bias⁶³, or a self-reported variable from a questionnaire prone to responder bias.

The heterogeneous but vast amount of studies on benefits of breast-feeding have been summarised into systematic reviews and meta-analyses. Often breast-feeding has been categorised as any breast-feeding or not breast-fed in order to pool evidence. This may weaken any associations between the exposure of breast-feeding and measured health outcomes^{23,64}. On the other hand, reviews provide the opportunity to investigate for publication bias (whether the probability of a study being published depends on the outcome of the study) and also support generalisability⁶⁵.

One major review of evidence published before May 2006 was conducted by the US Department of Health, including 43 studies on health outcomes in infants, 43 studies on health outcomes in mothers, and 29 systematic reviews and meta-analyses covering approximately 400 studies⁶⁴. Another review published by the WHO was concerned with long-term infant health outcomes such as blood pressure, blood cholesterol, risk of obesity and Type 2 diabetes, as well as cognitive performance and included studies published up until March 2006⁶⁵. The Dutch Centre for Nutrition and Health also published a large review of evidence of original studies from the developed world up until 2005⁶⁶. In these large reviews, the studies included were carefully rated for quality, and the quality varied considerably across studies. The ESPGHAN report that was used to base their recommendation on breast-feeding, incorporated these three reviews as well as other reviews and studies in a critical analysis of evidence published up to the end of 2008³². Finally, an update of the Cochrane review which formed the basis for the WHO recommendation was published in 2009, including studies up to December 2006⁴⁴. This and some of the most recent evidence will be the basis for this summary of risks and benefits of breast-feeding described below.

Risks of exclusive breast-feeding

Concerns have been raised, that exclusive breast-feeding to 6 months of age may increase the risk of growth-faltering, where the infant drops two or more centile lines below their baseline over a month or more^{67,68}. The updated Cochrane review by Kramer and Kahuma found that the evidence did not reveal any deficits in weight or length gain during exclusive breast-feeding to 6 months, but sample sizes were too small to detect risks of modest under-nutrition⁴⁴.

There may also be a risk for susceptible infants to develop iron deficiency anaemia during exclusive breast-feeding to 6 months due to the low iron content of breast milk. The updated Cochrane review found that infants born in developing countries with sub-optimal iron stores from birth may develop anaemia, if they were not supplemented with iron⁴⁴. Similarly the review on adequacy of exclusive breast-feeding by Butte⁴² concluded that infants not fed an exogenous source of iron after 6 months may be at risk of developing anaemia, but the evidence base was insufficient to quantify this risk at a population level⁴².

In a randomised controlled trial (the Honduras trial) of promotion of exclusive breast-feeding to either 4 or 6 months, they found that infants introduced to complementary foods at 4 months had higher iron intake as well as higher haemoglobin, haematocrit, and serum ferritin values than exclusively breast-fed infants. However, only exclusively breast-fed infants born <2500 g were at risk of anaemia and low serum ferritin⁶⁹. A study from the US found that infants breast-fed fully for 6 months or more (occasionally received other foods/liquids) had a higher frequency of parental reports of anaemia, but the study had methodological weaknesses⁷⁰. A study from Mexico with a better methodology, including measured haematology status at 6 months of age, found that predominantly breast-fed infants had a higher risk of iron deficiency compared to partially breast-fed and formula-fed infants, but there was no significant difference in the prevalence of anaemia⁷¹. In Scandinavian countries infants over 6 months of age are supplemented with iron drops (available from supermarkets) if they are not receiving iron-fortified complementary foods or formula^{72,73}, and such a practice could feasibly be implemented more widely and for younger infants considered to be at risk of iron deficiency.

Cases of hypernatraemic dehydration in exclusively breast-fed newborns have been published⁷⁴⁻⁷⁶. Hypernatraemic dehydration is a potentially very serious condition, which develops when the infant is feeding inadequately (lactation failure), and therefore develops dehydration with associated osmolar imbalance⁷⁷. Symptoms of hypernatraemic dehydration include failure to regain sufficient weight (initial weight loss >10% of body weight, or not having regained weight by 10 days post-partum) and lack of or insufficient urinary output⁷⁸. One study found that surveillance of newborns with early weighing (72 - 96 hrs post-partum) were associated with a reduced severity of hypernatraemic dehydration⁷⁸, possibly because lactation failure was diagnosed earlier.

Some critics have raised the concern that delaying the introduction of complementary foods to 6 months may risk that infants develop into toddlers with fussy eating behaviour. One retrospective study from 2011 in 139 pre-school children found that the children that were exclusively breast-fed to 6 months of age had 70 - 80% lower risk of developing neophobia and food rejection behaviour, but overall the evidence is conflicting⁷⁹. Evidence from a cohort of 7821 children found that those introduced to complementary foods after 9 months of age, showed more feeding problems at the age of 7 years, than those introduced to complementary foods between 6 and 9 months of age⁸⁰.

Finally, there are concerns regarding the effect of environmental toxins and organic pollutants that are transferred into breast milk, and may affect infant development and health⁸¹. There are also other endocrine disruptors, which do not figure as pollutants but nevertheless may affect infant health when present in breast milk⁸². With improved analytical capabilities, new publications of studies finding these compounds in breast milk are published frequently⁸³. However, the dose of exposure is often negligible for the individual infant⁸⁴, and when balancing the potential risk of these compounds against the benefits of breast-feeding over formula feeding, the general conclusion is that the benefits of breast-feeding by far outweigh the risks⁸⁵.

Benefits for the infant

The benefits of breast-feeding are both immediate and long-term, where long-term benefits may have weaker associations (require high power/large sample sizes) because of the impact of other modifying factors, which can have a variety of effects in different directions.

There is now a fairly solid base of evidence showing a short-term benefit in a reduced risk of infections from breast-feeding. The evidence is strongest for gastrointestinal disease and acute otitis media, and less strong, but still plausible for other respiratory infections^{30,32,64,86-89}. Some evidence indicates that this effect is more pronounced when infants are breast-fed exclusively^{29,90,91}, and one study found a dose-response relationship between the degree of breast-feeding and the risk of infections⁵⁷. A recent study from Brazil found no significantly increased protection of exclusive versus predominant breast-feeding, but significantly higher infection rates in infants that were partially breast-fed⁹². Another recent prospective study from Greece confirmed this finding⁹¹. This latter study had several strengths; breast-feeding was defined according to WHO, the rate of exclusive breast-feeding at 6 months was relatively high (25%), and the cohort was a large representative sample⁹¹.

The mechanism behind the protection from infections has been explained by the presence of secretory IgA antibodies, lactoferrin, and oligosaccharides in breast milk⁹³. The IgA antibodies are specifically targeted towards microbes in the maternal environment and therefore offer protection to the infant that lives in that same environment. Lactoferrin is a protein that can help in the destruction microbes, and oligosaccharides can block the attachment of microbes to the infant's mucosae and thereby prevent them from invading and cause infection⁹³.

Furthermore, breast milk may offer immunological benefits, which lower the risks or delay the development of allergies and diseases such as celiac disease, inflammatory bowel disease, Crohn's disease, and Type 1 diabetes in later life^{32,64,66,94}. For asthma, wheezing, and eczema there may be a protective effect of exclusive breast-feeding for at least 4 months - particularly in infants at risk due to a family history of these diseases^{64,66,95}. With regards to food allergies, the evidence is conflicting⁹⁶, but the theory that maternal dietary restrictions may help is not supported by evidence^{95,97}. A recent meta-analysis of 22 studies found no benefit of exclusive breast-feeding for 3 months to the risk of developing atopic dermatitis⁹⁸. Compounds in breast milk interact with the maturing infant immune system, affecting the development of tolerance as well as counteracting pro-inflammatory responses^{93,99}. In mice, development of tolerance starts during pregnancy, suggesting that it may be counterproductive for mothers to avoid allergens during pregnancy¹⁰⁰.

Several cardiovascular benefits have also been suggested. Studies have found associations between breast-feeding and a lower blood pressure in later life, although the magnitude of this benefit may be small and therefore of limited importance^{32,65}. The lipid profile may also be affected by breast-feeding, and blood total cholesterol is probably higher during infancy¹⁰¹, but lower in adulthood, in breast-fed versus formula-fed infants^{65,102,103}. One large cohort found higher high-density lipoprotein cholesterol in adults that were breast-fed as infants¹⁰⁴. However, there is evidence of publication bias among studies⁶⁵, earlier published meta-analyses did not sufficiently control for confounders, and several studies have not been able to confirm any benefit of breast-feeding on cardiovascular risk factors⁶⁴. Overall there is no convincing evidence of a beneficial effect on the risk of cardiovascular morbidity and mortality³².

A meta-analysis of 7 studies found a risk reduction of developing Type 2 diabetes in adolescents and adults who were breast-fed versus formula-fed (OR 0.61, 95% CI 0.44 to 0.85), but only three of the studies controlled for confounders¹⁰⁵. Insulin levels may be higher in formula-fed infants, and this could result in insulin resistance^{65,106}. Insulin resistance has also been linked to low levels of insulin growth-like factor 1 (IGF-1), which help support pancreatic β -cell mass and is influenced by early nutrition¹⁰⁷.

Many studies suggest that breast-feeding may offer a protection from childhood overweight and obesity^{32,64,65,108}. The pooled evidence from four meta-analyses^{59,109-111} - one of which revealed a dose-response relationship, where the risk of later obesity fell by 4% per month of breast-feeding⁵⁹ - produced an odds ratio of 0.78 (95% CI: 0.72 to 0.84) for being overweight or obese when breast-fed as infant⁶⁵. However, this study was criticised for an inadequate search-strategy, and for concluding on evidence that was compromised by publication bias and lack of control for confounding¹¹². More recently, a Swedish cohort study found that although exclusive breast-feeding for <4 months was associated with higher odds of obesity at 5 years, this was no longer significant, when controlling for confounders¹¹³. Similar lack of association with later body mass index (BMI), adiposity, overweight or obesity was also demonstrated in a cohort study from Australia¹¹⁴, two studies in the US^{115,116}, in studies from Kuwait¹¹⁷ and Brazil¹¹⁸, and in two cohorts in Asian populations^{119,120}. One analysis of a large cross-sectional sample found the significance of associations to depend on the scale of the variables (binary or continuous) and the statistical methods used¹²¹.

There are several suggested mechanisms for a link between breast-feeding and lower risk of obesity^{122,123}. For instance, it is possible that breast-fed infants learn to self-regulate their energy intake better¹²⁴⁻¹²⁷. Whereas bottle-feeding provides an opportunity for the mother to assess milk consumption and respond with behaviour that encourages overconsumption, the breast-feeding mother has to trust the infant's behaviour based on its own satiety cues¹²³. Formula-fed infants have energy intakes that are 15 - 23% higher than breast-fed infants⁹⁹.

Another possible mechanism is explained by “the early protein hypothesis”, which states that breast-fed infants' lower protein intake from breast milk protects against a high weight gain during infancy¹²². High weight gain during infancy has been positively associated with higher adiposity¹²⁸, and increased risk of overweight or obesity in childhood¹²⁹ and adulthood¹³⁰, or indeed at any age later in life^{131,132}. One study using the four-component model to measure body composition found relative weight gain from 0 - 3 months and from 3 - 6 months to be positively associated with fat mass (FM), waist circumference and trunk fat in childhood/adolescence¹³³. In general, formula-fed infants have protein intakes that are 66 - 124% higher than that of breast-fed infants^{99,134,135}.

Intake of protein stimulates insulin secretion¹³⁶ - also in infancy¹³⁷ - and insulin is an anabolic hormone that also promotes adipose tissue deposition. Different hormonal responses to feeding modes, including lower insulin levels of breast-fed infants, has been demonstrated¹⁰⁶. One study found a positive association between protein intakes in early life with childhood BMI¹³⁸, where protein intakes were high. Therefore, both higher intakes of protein and energy may contribute to the observed differences in growth patterns between breast-fed and formula-fed infants¹³⁹⁻¹⁴⁸, which may help to explain the associations with later obesity risk¹⁴⁹. The early protein hypothesis was tested in a large multinational double-blind randomised controlled trial in formula-fed infants, indicating that infants fed formula with a reduced protein content, mimicking that of breast milk, had a growth pattern close to that of breast-fed infants during the first 2 years¹⁵⁰. However, even if breast-feeding may have a protective effect against later obesity risk, this effect is probably small in size compared to other early life factors involved in the aetiology of obesity¹⁵¹. Finally, it has also been proposed that hormones present in human milk may influence infant energy metabolism, including appetite, milk intake, growth and body composition, with long-term consequences for infant health¹⁵².

A final infant benefit to consider is the possible effect of breast-feeding on cognitive development and intelligence^{32,64-66}. Breast-feeding has been associated with higher Intelligence Quotient (IQ) in childhood and adolescence - even when controlling for socio-economic status and maternal intelligence¹⁵³. The issue of residual confounding due to socio-economic status has been addressed in a study from the Philippines, where higher breast-feeding prevalence is associated with lower socio-economic status, but still found higher values of IQ¹⁵⁴. However, a more recent study found no significant association between breast-feeding and childhood intelligence after adjusting for confounders¹⁵⁵.

Mechanisms that have been proposed include the effects of the unique fatty acid composition of breast milk with a high concentration of long-chain polyunsaturated fatty acids (LCPUFA)¹⁵⁶. LCPUFA plays an important role in the maturation of the brain and retina. One population study found a positive association between maternal fish intake and the attainment of developmental milestones, which was independent of the positive association with breast-feeding duration¹⁵⁶. However, the impact of this benefit may be too small to have any real importance depending on the circumstances.

Nutritional Programming

Early infancy is seen as a period of great plasticity during maturation, where breast-feeding is considered the optimal way to guide this maturation towards short- and long-term health. An often used term is early nutritional programming (or imprinting), where exposures during limited, sensitive time periods of early development induce effects on endocrine, immunological and epigenetic processes, which then affect life-long health. Theories of nutritional programming¹⁵⁷⁻¹⁵⁹, like the early protein hypothesis¹²², are often developed from epidemiological studies. Subsequently, the mechanisms by which breast-feeding exerts benefits to infants are explored in animal studies. Among these studies, piglets appear to be a particularly good model for the human gastrointestinal track, which plays a crucial role in metabolic imprinting¹⁶⁰. Once theories are substantiated with evidence on the underlying mechanisms, it makes the benefits of breast-feeding somewhat plausible, even though the evidence in humans may be ambiguous.

Benefits for the mother

The suggested immediate benefits of exclusive breast-feeding for the mother include a delayed return of fertility (lactation amenorrhea)^{44,161}, but adherence to the definition of exclusive breast-feeding is important in relation to the risk of ovulation¹⁶². A better post-partum weight loss has also been observed¹⁶³, although the effect size is highly variable across studies with different breast-feeding definitions¹⁶⁴. Some studies have been criticised for their methodology and for claiming a benefit which may be small and without clinical importance⁶⁴.

The suggested long-term benefits include lower risk of rheumatoid arthritis, protection from pre-menopausal breast cancer, where a dose-response association has been found, and ovarian cancer, and several mechanisms for these benefits have been proposed^{64,66,165}. However, a systematic review of 30 studies concluded that only few studies found a protection against breast cancer from breast-feeding¹⁶⁶. A reduced risk of developing Type 2 diabetes has also been suggested⁶⁴, as insulin sensitivity is positively associated with sustained breast-feeding¹⁶⁷. Finally, more than 12 months of any breast-feeding seems to reduce the odds ratio of cardiovascular disease and associated risk factors¹⁶⁸.

The benefits of breast-feeding differ with provenance

The value of the benefits for infant and mother differ with provenance. For instance the protection from infections is a highly valued benefit in developing countries where infections are major causes of infant morbidity and mortality^{169,170}. Evidence from developing countries suggests no difference in protection from mortality between exclusive and predominantly breast-fed infants, whereas partially breast-fed infants are not as well protected¹⁷¹. Furthermore, some developing countries struggle with the risk of viral transfer from human immunodeficiency virus (HIV) positive mothers, but evidence from recent randomised controlled trials and cohorts suggests a reduced mortality and infectious morbidity in HIV exposed infants that are exclusively breast-fed to 6 months of age¹⁷². The theory of the “weanling dilemma”, where the benefit of protection from infection through prolonged exclusive breast-feeding has to be weighed against the risk of compromised infant growth due to nutritional insufficiency from breast-feeding alone¹⁷³, has been deemed unfounded⁴⁴.

In the developed world, there is readily available access to safe and nutritious alternatives to breast-feeding. Therefore, some immediate benefits are not so clear and profound. However, several recent studies conclude that also developed countries benefit from for instance fewer infections in exclusively breast-fed infants^{64,66,174}.

Confounding in studies also differs with provenance^{65,175}. In the developed world, breast-feeding prevalence is positively associated with affluence, implicating an environment with favourable health outcomes, which then overestimate the benefits of breast-feeding. Conversely, in the developing world breast-feeding is more prevalent among the poor, and the effect of deprivation may lead to an underestimate of the beneficial effects of breast-feeding⁶⁵.

In summary, there seem to be many potential benefits of breast-feeding - and also of breast-feeding exclusively - and the present description above is not even fully comprehensive. Even when these potential benefits may not reveal themselves at the individual level, they may still have a profound effect at a population level, if a high prevalence of breast-feeding is achieved^{176,177}. Despite this, breast-feeding prevalence is low in many countries, and often falls short of national targets⁸.

1.1.4 Prevalence of breast-feeding

Global and European prevalence in breast-feeding

In 2005, UNICEF found a global breast-feeding prevalence of 39% exclusive breast-feeding until 6 months¹⁷⁸. Data from the WHO “Global Data Bank on Breast-feeding” indicated in 2004 that 94% were ever breast-fed (any breast-feeding), 41% were exclusively breast-fed to 4 months, and 25% were exclusively breast-fed to 6 months (based on 195 countries)⁵. However, breast-feeding prevalence varied greatly both between and within countries.

Within the European countries the prevalence of breast-feeding has been slowly increasing, based on data collected in the 1990's^{10,179}, but also here, there are great variations both between and within countries¹⁰. It was noted that the prevalence of exclusive breast-feeding was only reported for the countries with high rates of any breast-feeding, and the use of breast-feeding definitions were very variable and not always well-defined¹⁰.

The Euro-Growth Study sampled 2245 infants from 12 cities between 1990 and 1993¹⁷⁹, and found the lowest breast-feeding rates in Toulouse, France and in Glasgow, UK. However, they did not ascertain a representative sample, and had a relatively high drop-out rate (24%), which was selective for breast-feeding. Overall, the rate of exclusive breast-feeding was 52% at 1 month and 3% at 6 months¹⁷⁹.

More recently, Cattaneo and colleagues included breast-feeding prevalence in European countries as part of a review of the status of implementation of the WHO global strategy on infant and young child feeding^{8,180}. They found only slow improvements in the implementation of the WHO global strategy, and there were great diversity in the measurement of breast-feeding prevalence. Overall, no real improvement in breast-feeding prevalence was observed from 2002 to 2007. Breast-feeding rates in general fell short of both recommendations and national targets. The UK was found to have a relatively low prevalence compared to most other countries in Europe⁸.

Prevalence of breast-feeding in the United Kingdom

In the UK, national surveys on infant feeding have been conducted every five years since 1975 (1980 for Scotland and 1990 for Northern Ireland)¹⁸¹ (Table 1.1).

In the two most recent Infant Feeding Surveys from 2005 and 2010, the methodology included the categories any breast-feeding and exclusive breast-feeding^{181,182}, as defined by WHO². However, for the 2005 survey prevalence of exclusive breast-feeding at initiation and at 6 months was only reported for the whole of the UK. Data from the 2010 survey on prevalence of exclusive and any breast-feeding at 6 months has not been published at the present time¹⁸¹. The populations included un-clustered samples of around 20,000 births in 2005 and around 30,000 births in 2010. The methodology was questionnaires to the parent/carer when the infants were around 4 - 10 weeks, 4 - 6 months and 8 - 10 months. Data on breast-feeding prevalence was therefore based on retrospective self-reports.

The overall response rate (of the original sample) was 47% in 2005 and 52% in 2010^{181,182}, and was selective for socio-demographic factors. For instance, in the Infant Feeding Survey from 2005, mothers who responded to the survey were more likely to come from a high socio-economic status area than non-responding mothers¹⁸².

Table 1.1 Breast-feeding incidence and prevalence at 6 months (%) in the UK and Scotland based on the Infant Feeding Surveys.

Year	Age	UK		Scotland	
		Any	Exclusive	Any	Exclusive
1990	Initiation	62	-	50	-
	6 months	-	-	-	-
1995	Initiation	66	-	55	-
	6 months	21	-	19	-
2000	Initiation	69	-	63	-
	6 months	21	-	24	-
2005	Initiation	76	45	70	-
	6 months	25	<1	24	-
2010	Initiation	81	-	74	-
	6 months	-	-	-	-

Sources: Infant Feeding Surveys of 2005¹⁸² and 2010¹⁸¹.

The prevalence of both any and exclusive breast-feeding in Scotland was lower than the UK average. Both the 2005 and the 2010 surveys noted that breast-feeding prevalence was unevenly distributed with regional variation that reflected the regional variation in socio-demographic factors^{181,182}. In addition, both surveys found breast-feeding prevalence to be positively associated with maternal age and education level, and negatively associated with degree of deprivation. There was also a higher breast-feeding prevalence among non-smoking mothers and primiparous mothers^{181,182}.

Based on the Infant Feeding Surveys, breast-feeding prevalence has been increasing over time, but with concomitant changes in socio-demographic variables of the sample. If adjusted for this, the initiation rate for Scotland was 57% in 2005 and 58% in 2010. Hence, the changes observed in the initiation rates are more likely to reflect a change in the composition of the sample rather than a real change in incidence¹⁸¹. A similar conclusion was found in the 2005 survey, when reflecting on the changes between 2000 and 2005¹⁸².

The Avon Longitudinal Study of Parents and Children (ALSPAC) included data on breast-feeding from a large cohort of 11490 infants, representing 81% of all live births within the area of south-west England in 1992-1992. The prevalence of exclusive breast-feeding, as defined by WHO², was 54% at 1 month, and 0.4% at 6 months of age¹⁸³. The prevalence of any breast-feeding was 80% at 1 month and 36% at 6 months of age, which is a high prevalence of breast-feeding relative to the Infant Feeding Survey, reflecting the affluence of the area.

Prevalence of breast-feeding in Scotland

The Information Services Division (ISD Scotland) has published breast-feeding prevalence at around 10 days and 6 – 8 weeks of age for the health boards participating in the Child Health Systems Programme Pre-school system (CHSP-PS)¹⁸⁴ (Table 1.2). These data represent from 84% (2001/02) to 100% (2010/11) of the Scottish population of newborn infants, and is as such with less risk of sample selection bias. Overall breast-feeding incidence and prevalence are considerably lower than what is reported in the Infant Feeding Surveys, and have remained fairly static in recent years, while it was increasing in the 1990's^{185,186}. Also within Scotland, breast-feeding prevalence vary with geographical area, where City of Glasgow has a breast-feeding prevalence lower than the Scottish average. Breast-feeding was positively associated with maternal age and educational level, and negatively associated with degree of deprivation¹⁸⁴.

Table 1.2 Breast-feeding incidence (10 days) and prevalence at 6 – 8 weeks of age (%) in Scotland and City of Glasgow.

Year	Age	Scotland		City of Glasgow	
		Any	Exclusive	Any	Exclusive
2001/02	10 days	43.5	39.0	38.8	33.6
	6 – 8 weeks	35.7	26.9	32.6	23.9
2002/03	10 days	44.5	39.5	40.9	35.2
	6 – 8 weeks	36.5	27.5	34.0	24.9
2003/04	10 days	44.3	39.0	39.0	33.2
	6 – 8 weeks	35.8	27.1	33.3	24.4
2004/05	10 days	44.4	38.6	40.6	34.5
	6 – 8 weeks	36.3	27.2	34.6	25.6
2005/06	10 days	44.7	38.3	40.7	33.3
	6 – 8 weeks	36.8	27.9	35.4	26.1
2006/07	10 days	44.1	37.5	42.7	34.3
	6 – 8 weeks	35.7	26.2	35.3	24.6
2007/08	10 days	44.6	37.2	41.6	32.6
	6 – 8 weeks	35.5	26.2	33.6	23.3
2008/09	10 days	45.4	37.2	44.4	33.0
	6 – 8 weeks	36.2	26.6	36.2	24.3
2009/10	10 days	45.6	36.3	45.5	33.0
	6 – 8 weeks	36.0	26.1	37.4	24.7
2010/11	10 days	46.8	36.3	46.9	31.9
	6 – 8 weeks	37.1	26.5	37.5	25.4

Source: ISD Scotland¹⁸⁴.

Data such as these, and the Guthrie cards^{187,188}, are probably more accurate and representative for measuring breast-feeding incidence and prevalence. However, it is often the Infant Feeding Surveys that are cited in the literature. In summary, the First-Feed study was conducted in West-Central Scotland, an environment of very low breast-feeding prevalence and the sample of participants was therefore not expected to be representative of Scottish mothers.

1.1.5 Determinants of breast-feeding

The literature looking into factors associated with breast-feeding incidence and/or prevalence is very diverse, and it is beyond the scope of the present thesis to provide a full account of this topic. Instead, this section will condense some of the findings from recent studies from the Western world with references to further reading.

The factors associated with breast-feeding can be considered confounding factors, which needs to be adjusted for (section 1.1.3), but they can also be regarded as predictors of the risk of not breast-feeding or early cessation of breast-feeding¹⁸⁹⁻¹⁹². For this reason these factors are also termed *determinants* or *influencing factors* of breast-feeding in the literature^{193,194}. Studies identifying these factors are important because they help direct promotional efforts to the populations where these efforts are most needed, and they inform the development of strategies for these promotion efforts to optimise their effect¹⁹⁴. The overall goal would be to tackle the social inequalities related to infant feeding¹⁹⁵. The studies vary greatly in population circumstances (e.g. focusing on primiparous¹⁹⁶, adolescent¹⁹⁷ or deprived¹⁹⁸ mothers and from different countries) and in the research design and methodology employed (including unclear definitions of breast-feeding). Although most factors influence both incidence and prevalence of breast-feeding, they may vary in their relative importance for breast-feeding initiation, duration and also for exclusivity. Factors associated with breast-feeding prevalence include demographic, biological, social and psychological factors¹⁹⁹, but many of these factors act as confounders²⁰⁰ which complicate the interpretation of the results as they are often erroneously entered into one multivariate statistical model²⁰¹.

Factors important for breast-feeding incidence

Initiation of breast-feeding precedes breast-feeding for an extended duration. One factor strongly associated with initiation of both any²⁰²⁻²⁰⁴ and exclusive breast-feeding²⁰⁴ seems to be the antenatal intention to breast-feed, which is itself influenced by many factors, including socio-demographics²⁰⁵, maternal characteristics, knowledge of benefits of breast-feeding¹⁹⁰ and other health behaviours²⁰⁶, as well as birth complications¹⁹⁸ and previous breast-feeding experience^{200,207}. In a study using the same questionnaire as in the First-Feed study (section 2.6.2), the intention to breast-feed exclusively depended on maternal knowledge of the benefits of breast-feeding and on comfort with breast-feeding in public¹⁹⁰. Studies using the theory of planned behaviour (see below) have found the intention to breast-feed exclusively to be a strong predictor for both initiation and duration of exclusive breast-feeding^{198,208,209}.

Socio-demographic factors

Various markers of socio-economic status are associated with breast-feeding initiation, duration and exclusivity. In section 1.1.4, it was described how both data from the CHSP-PS and the Infant Feeding Surveys found socio-demographic differences in breast-feeding prevalence relating to degree of deprivation^{181,182,184}. This is also the case if socio-economic status is measured as family income^{189,193,195,199,207,210}, or in the UK as Index of Multiple Deprivation (IMD)¹⁹¹ or council tax evaluation band²¹¹. For instance, in the Gateshead Millennium study they found that the most affluent mothers were three times more likely to initiate breast-feeding and five times more likely to be breast-feeding at 4 months post-partum than the most deprived mothers¹². Other studies have found urbanisation (measured as numbers of inhabitants in the city/village) to be associated with breast-feeding prevalence with higher breast-feeding prevalence in suburban areas than in rural areas²¹². Ethnic differences in breast-feeding initiation, duration and exclusivity have also been explored²¹³⁻²¹⁶.

Maternal factors

Data from the CHSP-PS and the Infant Feeding Surveys found strong positive associations between breast-feeding prevalence and factors such as parity, maternal age at childbirth and education level^{181,182,184}.

A review¹⁹³ as well as more recent studies from for instance Canada^{189,217}, the US^{218,219}, Australia¹⁹², Ireland²²⁰, Germany²²¹, Netherlands²²² have revealed similar findings. Maternal health-related behaviours are also associated with breast-feeding prevalence. For instance, non-smoking mothers are more likely to initiate and sustain breast-feeding^{189,192,200,205,221,223-226}, while maternal overweight and obesity is associated with lower rates of initiation²²⁷ and shorter duration²²⁸ of any breast-feeding²²⁹. It was noted by Amir and colleagues that although a dose-response relationship has been documented between maternal BMI and breast-feeding prevalence²³⁰⁻²³², it may not be evidence of a causal relationship²³³.

Many determinants of breast-feeding, like the ones mentioned here, are not easily modifiable, and for those factors it is more important to have sufficient screening tools to identify mothers at risk, whilst changing the actual determinants themselves is a different priority.

Behavioural factors

Whilst the determinants described above are not easily modifiable, other factors associated with breast-feeding are more related to cultural environments, perceptions and attitudes, some of which may be modifiable through appropriate breast-feeding education and support. Such factors are in the present thesis termed *behavioural factors* (Chapter 7).

Studies using the theory of planned behaviour have found that both the partner's and health professionals' attitudes to breast-feeding are important for breast-feeding success^{198,208,209,234}. In brief, the theory of planned behaviour states a setting, where for instance the mother's ultimate choice of infant feeding is a result of 1) the mother's own attitude to breast-feeding, 2) the perceived social pressure or acceptance of breast-feeding (subjective norm) and 3) the mother's perception of her ability to breast-feed (perceived behavioural control)¹⁹⁸. While the mother's own beliefs may be more important for the intention to breast-feed, the subjective norms become more important for infant feeding decisions post-partum²⁰⁹, and also for the decision to persevere with exclusive breast-feeding to 6 months²³⁴. Other studies using questionnaires²³⁵, focus groups or individual interviews have also reported that paternal attitude and/or family support^{236,237} as well as adequate support and encouragement from health care professionals^{220,238,239} is important for breast-feeding prevalence.

This very condensed overview only mentions a few of the many factors associated with breast-feeding prevalence, but the fact that they are so strongly and consistently associated may imply that such factors are more important than lactation physiology per se. They may determine which infant feeding practice the mother ultimately adopts; whether she breast-feeds or not, whether she does so exclusively, and also for how long. Given the low prevalence of breast-feeding in the central belt of Scotland, and given the necessary inclusion criteria of exclusive breast-feeding (section 2.2.2), the First-Feed study was not expected to ascertain a representative sample of Scottish mothers. This issue will be addressed in chapter 3, where the sample of participants is characterised.

1.2 Energy balance during exclusive breast-feeding

This section will describe the terminology and definitions of infant energy balance, including energy expenditure, energy intake and energy requirements, as well as the principles of how these variables can be measured. There is also an overview of infant milk intake and how this can be measured. Finally, there is a review of the current evidence base on milk intake in exclusively and predominantly breast-fed infants.

1.2.1 The principles of measuring energy expenditure

Energy and calorimetry

The first law of thermodynamics, stated by Lavoisier, claims that no energy can be created or destroyed, but it can change from one state to another²⁴⁰. This is the basic assumption upon which the study of infant energy balance in the present thesis lies. Energy in its simplest state is heat, and heat production is measured by *direct calorimetry* when a fuel is combusted. For instance, the heat energy emitted when breast milk is combusted in a bomb calorimeter²⁴¹ (breast milk ignited under high pressure in presence of oxygen), makes the value of *gross energy content* of that breast milk (section 1.2.3). Whenever fuel is combusted, energy is released and expended, O₂ (hereafter oxygen) is used and CO₂ is produced.

Instead of measuring energy as heat production, it can be determined from the rate of oxygen consumption, V_{O_2} , and/or CO_2 production, V_{CO_2} , by *indirect calorimetry*. The oxygen used and CO_2 produced from combustion of a fuel in the body depend on the fuel, i.e. the macronutrient composition and quality. This can be taken into account by the *respiratory quotient*, RQ , which is the ratio of V_{CO_2}/V_{O_2} under *steady state*, where the oxygen supplied equals the oxygen required by the tissues for the combustion of the fuel (i.e. no anaerobic metabolism). One study measuring RQ in exclusively breast-fed infants found a value of 0.94 at 1 month and 0.90 at 4 months²⁴², while it is typically 0.85 in adults on a Western diet. Oxygen consumption and CO_2 production can be determined using *closed circuit methods*, e.g. in respiration chambers, where the subject's breathing is isolated from the outside air, or *open circuit methods*, where a flow of atmospheric air is passed by the subjects head and changes in the concentrations of oxygen and CO_2 are measured. When the combustion of protein is negligible (only carbohydrates and fats used as fuel), the energy expenditure is calculated according to Weir's equation:

$$\text{energy expenditure} = V_{O_2}(3.941 + 1.106RQ) \text{ (kcal)}^{243}.$$

Both open and closed circuit methods are used in the laboratory, and this unfamiliar environment may influence the measurements. Moreover, they are not suitable for measuring energy expenditure over a longer period of time, in order to increase precision by accounting for within-day and between-day variability. Therefore, the ideal would be to measure total energy expenditure (TEE) over a longer period of time under free-living conditions, and for this the DLW method is the criterion method. To introduce the DLW method, the principles on which this method lies are described here (see also Appendix B).

Isotopes in water and water flux

The atoms that make water can exist as different isotopes. An isotope is any form of an atom with the same atomic number, but different atomic weights. Hydrogen can exist in three isotopic forms, 1H (protium), 2H (deuterium), and 3H (tritium) while oxygen exists in the isotopic forms; ^{16}O , ^{17}O , and ^{18}O (oxygen-18). Both deuterium and oxygen-18 are stable isotopes, which mean they don't emit any particles by radiation. Given that they have the same electron structure as their lighter isotopic forms, they also have the same chemical properties.

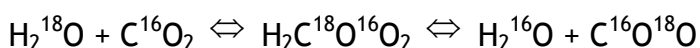
They are present everywhere in abundance levels (typical ratios are $^2\text{H}/^1\text{H} = 0.00014$ and $^{18}\text{O}/^{16}\text{O} = 0.00204^{240}$) (Appendix B.1). In water, deuterium and oxygen-18 can exist in a number of combinations ($^{16}\text{O}^1\text{H}^1\text{H}$, $^{16}\text{O}^1\text{H}^2\text{H}$, $^{17}\text{O}^1\text{H}^1\text{H}$, $^{18}\text{O}^1\text{H}^2\text{H}$ etc.). Some of these are very rare, and the mass spectrometer can correct for variations in the common molecular compositions of water to give an accurate ratio of each of the isotopes. Given in an amount above the abundance levels, heavy isotopes can be used to trace the journey of different molecules through the body, and also the rate with which they are eliminated.

The *water influx* in infants that are exclusively breast-fed according to the WHO definition² comes primarily from breast milk. Additionally, some water enters as environmental water influx absorbed from the air either transcutaneously or through the lungs^{244,245}, and processes of metabolism also create water influx, which is either eliminated or used elsewhere.

The *water efflux*, or elimination, is evaporation through lungs and skin, as well as excretions and secretions. The average time it takes from a water molecule entering the body until it is eliminated again, is the *water turnover*. Exclusively breast-fed infants have a high water turnover due to their liquid diet and their high metabolism relative to their size^{246,247}. The term *biological half-life* can be used to describe the turnover rate and is defined as the time it takes for half of a given dose of for instance labelled water to be eliminated again. The biological half-life of water in infants can be calculated to around 2.6 days²⁴⁸. The water in the body (total body water, TBW) is distributed almost exclusively in the fat free mass (FFM). Roughly 79% of FFM is water²⁴⁹, while TBW as percentage of body weight changes throughout infancy²⁵⁰ due to changes in body composition. Body water may be used in metabolism that leads to energy being expended, it may be stored in tissues, or it may be eliminated.

The principles of the doubly-labelled water method

The idea behind the DLW method was originally developed in the late 1940's and early 1950's by Professor Nathan Lifson and colleagues after having discovered that the oxygen in H_2O and CO_2 is in equilibrium²⁵¹, due to the activity of the carbonic anhydrase, an enzyme present in red blood cells, in the lungs and elsewhere:



Because of this equilibrium, deuterium is eliminated through water efflux only, while oxygen-18 is eliminated both through efflux of water and through expiration of CO₂. Therefore the elimination rate of oxygen-18 is higher than that of deuterium and the difference between these elimination rates corresponds to V_{CO2}. By administering concentrated DLW to a subject and measuring the exponentially declining concentrations (elimination rates) of deuterium and oxygen-18 in urine samples, it is possible to determine the average V_{CO2} over the elimination period. V_{CO2} can then be used to calculate how much energy has been expended (TEE) using the Weir's equation²⁴³. The DLW method, as it was used in the First-Feed study, will be described in detail in section 2.3, and the underlying assumptions and corrections for water fluxes and fractionation are described in Appendix B.

1.2.2 Infant energy balance and energy requirements

Principles of energy balance

When a body is in steady state in terms of energy (in energy balance), the metabolisable total energy intake (hereafter TEI refers to metabolisable total energy intake) is equal to TEE²⁴⁰. But as implied above, infants are not in a steady state, but are gradually accumulating body mass (in positive energy balance). Hence TEI of healthy infants is equal to TEE plus the energy stored in new tissues (growth), E_{growth}.

Total energy expenditure

TEE includes basal metabolism (metabolism necessary for the essential functions and maintenance of tissues), thermogenesis (heat production due to e.g. digestion and cold exposure) and physical activity. For growing infants, it also includes the cost of synthesising new tissues. TEE is mainly determined by sex, age and body size (body weight and body composition)⁷. A study by Butte and colleagues found a within-individual variation in TEE of 18% in 3 - 24 month-old infants, while between-individual variation in TEE was about 19%²⁵².

The rate of basal metabolism (BMR) constitutes a large part of TEE in newborns, but declines during infancy as the body weight increases to include relatively more of less metabolically active tissues²⁵³. The value of thermic effect of feeding is about 10% of the basal metabolism in adults on a mixed diet⁷.

Calculated as the increase in energy expenditure from 0 - 2 hours after a feed (post-prandial) it has been found to be around 2 - 3% of TEE in exclusively breast-fed infants²⁵⁴. Physical activity can be very variable, and one study found that the fraction of energy intake that is spent on physical activity increased from about 5% at 1.5 months of age to about 34% at 12 months of age²⁵⁵.

Energy stored in growth

The *energy stored in growth*, E_{growth} - also termed the energy deposited in new tissues - is the amount of energy contained in the new tissues once they are built, but it does not include the cost of synthesising them. The *energy cost of growth* is the sum of E_{growth} and the cost of synthesising new tissues (part of TEE)²⁵⁶. The energy cost of growth constitutes about 35 - 40% of the energy intake in newborns, and then decreases to about 5% at 12 months⁷.

E_{growth} is mainly composed of fat and protein (carbohydrate is negligible). Butte and colleagues used the multi-component model, which includes separate measurements of TBW (from isotope dilution), total body potassium (determined using ^{40}K detectors) and bone mineral content (from dual-energy x-ray absorptiometry, DXA) to calculate growth in its components of fat and protein gains from 3 - 24 months¹⁴⁵. This study demonstrated how the rate of growth changes during infancy, and how the new tissues contribute to changes in body composition¹⁴⁵.

Energy requirements

According to the references for human energy requirements by the Food and Agriculture Organization of the United Nations, WHO and the United Nations University (FAO/WHO/UNU)⁷; "Energy requirement is the amount of food energy needed to balance energy expenditure in order to maintain body size, body composition and a level of necessary and desirable physical activity consistent with long-term good health. This includes the energy needed for the optimal growth and development of children..."⁷. These published human energy requirements are meant to be prescriptive at the group level to support and maintain health and good nutrition, as chronic energy imbalance increases vulnerability to infections and non-communicable diseases⁷. The criterion method for determining infant energy requirements is the factorial approach of adding measurements of TEE to estimates of E_{growth} .

Studies of energy intakes can be influenced by external factors and are more prone to inaccuracy and imprecision²⁵⁷. Furthermore, the energy intakes measured in populations - even if they were both accurately and precisely measured - do not necessarily represent a desirable intake to fulfil energy requirements according to the above definition²⁵². Although the factorial approach is the criterion method for determining infant energy requirements, it is done assuming that the observed growth and the estimated E_{growth} are optimal in relation to long-term health.

Energy requirements vary between-individuals and also from day-to-day within individuals⁷. One study found within-individual variation in energy intake in 1 - 5 months-old mainly partially breast-fed infants to be around 10%, while between-individual variation was around 20%²⁵⁸. Moreover, there does not seem to be any tight regulation between energy intake and energy expenditure - at least not in the short term (e.g. 7 days)²⁵⁹.

For breast-fed infants, energy requirements differ by age and sex, partly due to differences in FM and FFM²⁶⁰. If TEI is inadequate compared to energy requirements, growth may be affected. Hence growth can be considered an indicator of whether energy requirements are met²⁶⁰. For reference values of energy requirements, see section 6.1.2 (Table 6.1). Energy requirements were determined using the factorial approach in exclusively breast-fed infants in the First-Feed study and will be further discussed in Chapter 6.

1.2.3 Measuring breast milk intake and milk energy content

In exclusively breast-fed infants, as defined by WHO², the energy intake which fulfils energy requirements must come from breast milk alone. Scanlon and colleagues have reviewed studies assessing the validity of measuring milk intake in infants¹³. One way of estimating breast milk intake is direct observation, which may include observing characteristics of breast-feeding (infant latch, suckling rhythm as well as breast fullness) and/or timing the breast feeds and assuming an average milk flow across the length of each feed. However, this method is relatively crude, and does not predict breast milk intake well²⁶¹ showing decreasing accuracy with increasing milk volumes¹³. As better methods are available, the direct observation method will not be further discussed in the present thesis.

Test-weighing

Alternatively, breast milk intake can be measured by test-weighing, which is a method that has been extensively validated¹³. The test-weighing procedure typically involves weighing the infant before and after breast-feeding over a period of 24 to 48 hours. The difference in weights constitutes gross breast milk intake, but must be corrected for the insensible water loss (IWL) that has taken place during the feed²⁴². Provided it is done, the test-weighing procedure may provide a reasonably accurate and precise measurement of the gross milk intake¹³, which then needs to be corrected for metabolisability³ (see below).

However, the reliability of the test-weighing method to measure milk intake has been questioned, since many validation studies have only used correlations, which does not preclude poor precision and accuracy²⁶². Studies have found that the test-weighing method tends to underestimate milk intake^{3,13} (see below), and may be inaccurate in situations with small but frequent breast-feeds^{262,263}.

The test-weighing method has also been criticised for being intrusive on the breast-feeding routine as the mother needs to perform infant weights before and after each breast-feed (often also at night). This places a burden on the mother, and incurs a risk of responder bias; the mother changing infant feeding practices in response to the study. Additionally, test-weighing itself can be a challenge and if there are consistent differences in activity levels of an infant between before (probably active or even crying) and after (tired and perhaps subdued due to a full stomach) a feed, this could in theory also influence the measurements. Alternatively, the test-weighing can be done on the mother¹³, which is less intrusive for the infant, but still burdens the mother. Therefore, the test-weighing procedure is usually not performed over several days, which reduces precision at the individual level²⁶⁴. Different studies have used different weighing regimes, and circumstances may make it difficult for one standardised procedure to be applicable, reducing comparability across studies¹⁴.

Isotope kinetics for measuring milk intake

A less intrusive way of measuring milk intake is the deuterium dose-to-infant method, as first proposed by Coward and colleagues in 1979¹⁴. This method allows the mother to breast-feed according to her usual routines and does not require her to record any data.

The method involves giving the infant a carefully measured dose of deuterium-labelled water and take urine samples pre- and post-dose to determine baseline abundance, post-dose enrichments and elimination rate. To use this method, it is necessary to correct for water flux that is not due to breast milk intake^{244,265}. Any non-breast milk water intake will be calculated as breast milk and lead to an overestimation of breast milk intake if infants are not exclusively breast-fed.

The possible overestimation of breast milk intake from non-breast milk water intake can be avoided by using the deuterium dose-to-mother approach instead, whereby the infant becomes enriched with deuterium through the consumed breast milk only, which is then measured in urine samples of the infant²⁶⁶⁻²⁶⁸. Through deuterium kinetics model fitting it is possible to derive both the breast milk intake and intake of non-breast milk water^{28,269,270}. One draw-back of this method is the higher cost of analysis of samples from both mother and infant. The amount of milk intake measured using isotope kinetics is the *metabolisable milk intake*, since it is derived from post-absorptive variables.

Milk intake measured from deuterium kinetics was compared to test-weighing in breast-fed infants, in the original study by Coward and colleagues, where the isotopic method produced higher estimates of milk intake, possibly due to underestimation of the test-weighing method¹⁴. A study by Butte and colleagues also assessed the deuterium dose-to-infant method, and found significantly higher milk intakes compared to the test-weighing method, but they did not correct for IWL²⁷¹. In a study that included correcting for IWL, the deuterium dose-to-infant method still revealed higher milk intakes than 5-day test-weighing²⁷², and a study of the deuterium dose-to-mother method using test-weighing as the reference method revealed similar findings²⁶⁸. When the deuterium dose-to-infant method was validated in formula-fed infants against direct weighing of feeding bottles, the results were similar between the two methods^{245,265,273,274}, suggesting it may be the test-weighing method that can be prone to underestimation of milk intake²⁷³. As the DLW dose-to-infant method also involves deuterium kinetics, this method equals the deuterium dose-to-infant method for measuring metabolisable milk intake. The simultaneous measurement of weight and body composition allows an estimation of E_{growth} , and this value added to TEE provides the value of TEI, which is referred to as *metabolisable total energy intake* since it is calculated from post-absorption variables (section 1.2.1).

Milk energy content

When milk intake is quantified, energy intake can be determined from the energy content of breast milk, typically measured from milk samples. Energy content in samples of breast milk can be determined through bomb calorimetry²⁴¹, chemical analysis of macronutrients²⁷⁵, which provide measures of gross breast milk energy content. Other studies have used crematocrit values, as a proxy for fat content, which is measured as the relative size of the supernatant (lipid) in a capillary tube of breast milk after centrifugation²⁷⁶. All these methods need to be corrected for *metabolisability*, i.e. the fraction of energy available for metabolism.

Breast milk varies in composition (section 1.2.5), and different milk sample regimes have been employed in different studies with the objective of obtaining a representative milk sampling^{277,278}. However, it can never be ascertained if the milk sampling regime results in a representative sample.

Even during complete evacuation of the breast, there is no certainty that the energy expressed would be the energy alternatively retrieved by the infant²⁷⁹. The DLW method provides information on an average value over a week of metabolisable milk energy content (from here milk energy content refers to metabolisable breast milk unless otherwise stated) from the simple division of metabolisable TEI with the amount of metabolisable milk intake²⁷⁹. The DLW method therefore both deals with the imprecision caused by variability in breast milk, and with the correction for metabolisability.

1.2.4 Evidence of breast milk intake

The evidence of breast milk intake of exclusively (and predominantly) breast-fed infants is mainly included in two important reviews^{3,42,270}, three more recent publications, and one very newly published randomised controlled trial⁵⁴.

Report on nutrient adequacy of exclusive breast-feeding for 6 months

As part of the report on nutrient adequacy of exclusive breast-feeding (section 1.1.2), Butte and colleagues summarised evidence of milk intake from breast-fed infants⁴². The literature included mostly studies from the 1980s and 1990s. For the developed world, 17 of the 22 studies compiled had measured milk intake using the test-weighing method (Table 1.3)⁴².

The pooled evidence showed milk intake tended to increase gradually throughout infancy from 699 g/d at 1 month⁴². They also noted that boys consumed more milk than girls and that girls tended to be exclusively breast-fed for longer²⁸⁰.

Table 1.3 Milk intakes (mean \pm SD) for the developed and the developing world.

	Developed world		Developing world	
	mean \pm SD	n	mean \pm SD	n
3 months, g/d	751 \pm 130	376	582 \pm 42	34
4 months, g/d	780 \pm 138	257	768 \pm 63	95
5 months, g/d	796 \pm 141	131	778 \pm 83	97
6 months, g/d	854 \pm 118	93	804 \pm 76	64

Source: Butte and colleagues⁴²

The evidence from the developing world included five studies⁴², and revealed milk intake as somewhat lower than the evidence from the developed world. However, Butte and colleagues included both exclusively and predominantly breast-fed infants in their review. Hence this summary of evidence includes infants that were not breast-fed exclusively as defined by WHO².

Although the majority of the findings from these studies were obtained using the test-weighing method and therefore providing gross intakes of breast milk, the review did not describe in detail how corrections for IWL and metabolisability were dealt with⁴².

Systematic review of milk intake in exclusively breast-fed infants

Following the implementation of the WHO recommendation of exclusive breast-feeding to 6 months in England⁴⁶, Reilly and colleagues conducted a systematic review of the evidence of milk intake from the developed world³ in infants that were strictly exclusively breast-fed, as defined by WHO². Here the search strategy was carefully described, including choice of bibliography data-bases and selection criteria for both study participants and methodologies used to obtain measurements of milk intake, and the literature was searched up until 2002. They applied an evidence based approach to systematically correct all gross measurements of milk intakes to metabolisable milk intakes by factoring in IWL and metabolisability, and they analysed cross-sectional and longitudinal studies separately³.

In all, they identified 33 cross-sectional studies of infants aged 3 - 4 months, 6 studies of infants at 5 months of age, and 5 studies of infants at 6 months of age (Figure 1.1). Among all the cross-sectional studies, 38 had used the test-weighing method, and three studies had used an isotopic method. Seven-teen of these studies were also included in the review by Butte and colleagues⁴². The weighted mean and standard deviation (\pm SD) milk intakes were 779 ± 40 g/d, 827 ± 39 g/d and 894 ± 87 g/d at 3 - 4 months, 5 months and 6 months, respectively. Additionally, Reilly and colleagues identified nine longitudinal studies, which all used test-weighing to measure milk intake, and had at least two measurements of milk intake in exclusively breast-fed infants, as defined by WHO². Collectively, the age-range in the studies was 2 - 5 months of age, but all nine studies reported no marked increase in milk intake during each study period. Finally, they performed a sensitivity analysis between 38 test-weighing studies and three isotopic studies, which revealed that metabolisable milk intake was significantly higher using an isotopic method than when using the test-weighing method (864 ± 63 g/d versus 799 ± 47 g/d, $P = 0.02$), although the isotopic studies were all conducted in 3 - 4 months old infants.

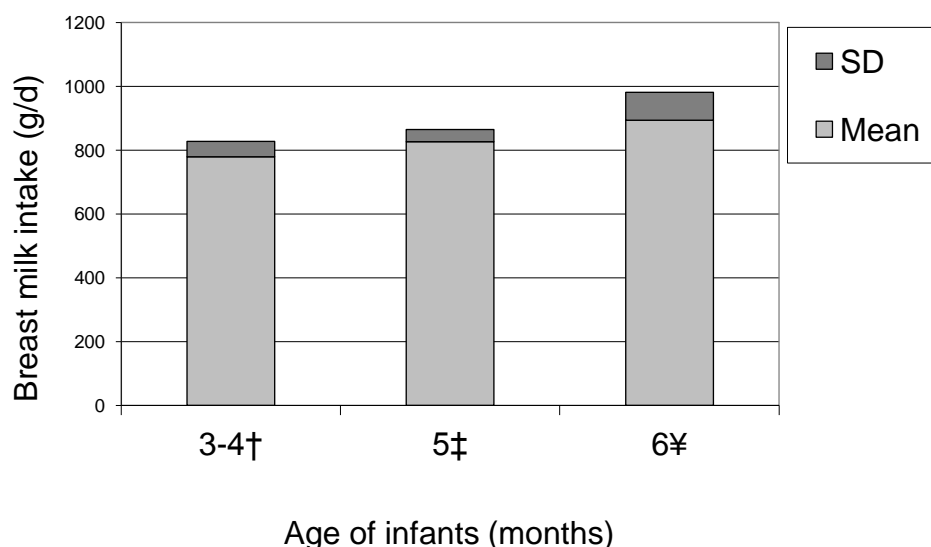


Figure 1.1 Compiled weighted mean milk intakes from cross-sectional studies.

† 33 studies, n = 1041 infants

‡ 6 studies, n = 99 infants

¥ 5 studies, n = 72 infants

Other recent publications

In the Dortmund Nutritional and Anthropometric Longitudinally Designed study (DONALD study), they had detailed dietary records of all infant dietary intakes (including 3-day test-weighing), and could thus reveal milk intake on infants that were fed breast milk plus tea and/or water²⁸¹. This study found milk intakes at 6 months of age that were very similar to the findings of the systematic review, and that milk intake had increased from 3 months to 6 months of age, but only significantly so in girls. If the measured milk intake per kg body weight was multiplied with the mean value of milk energy content found in the systematic review, the TEI of the breast-fed infants in the DONALD study²⁸¹ came close to the references for energy requirements as published by FAO/WHO/UNU⁷.

Da Costa and colleagues recently published a study that pooled data from studies using deuterium dose-to-mother to measure milk intake²⁷⁰. The data were pooled from 10 cross-sectional studies and six longitudinal studies, from both developed and developing countries and from both exclusively and predominantly breast-fed infants. Their data-analysis revealed a rapid rise in milk intake until 3 - 4 months of age, followed by a stagnation or slight decline until 8 - 9 months of age, possibly due to the introduction of complementary foods²⁷⁰. They also found that boys overall had a higher intake than girls by about 50 g/d, but the difference in milk intake between developed and developing countries was not clear (due to differences in age groups between studies from different countries)²⁷⁰.

Finally, a recent study was published in the present year in Cameroonian mothers, which reported a mean milk intake of 701 mL/d (equivalent to about 723 g/d) in infants between 1 and 4.5 months of age using the deuterium dose-to-mother method²⁸². Finally, the most recent randomised controlled trial found milk intake of exclusively breast-fed infants to be 901 ±158 g/d at 6 months of age. As it is a randomised controlled trial, this is the most reliable evidence on milk intake in exclusively breast-fed infants available to this date.

1.2.5 Evidence of milk energy content

Breast milk is very complex and variable in its composition of macronutrients, micronutrients and other bioactive components^{283,284}, and it is beyond the scope of the present thesis to include a general overview of breast milk composition.

This section will thus only introduce some evidence of milk energy content—especially in exclusively breast-fed infants. As fat is the most energy dense component of breast milk, the milk energy content is closely related to the fat content, but the fat content in breast milk is very variable²⁸⁵. Sources of the measured variation in breast milk fat and energy content include sampling methods and analytical methods (section 1.2.3), as well as biological variation in breast milk. Biological variation constitutes variation between-individuals²⁸⁶, within-feed^{285,287-289}, between-feeds^{288,290,291} and between-breasts^{285,288,292}, whereas there are conflicting reports with regards to evidence of an actual circadian rhythm^{285,288,293}. Some studies have found variations in gross milk energy content or fat content with duration of lactation^{285,288,293,294}.

In the systematic review by Reilly and colleagues, milk energy content was assessed from studies of exclusively breast-fed infants only³. The isotopic methods revealed an energy content of 2.5 kJ/g, while the mean of both isotopic methods and milk samples was 2.6 kJ/g. However, due to different sampling regimes in the different studies, it was not possible to assess if milk energy content was different in different age-groups, or if it changed over time³. When assessing the adequacy of exclusive breast-feeding, Butte and colleagues quoted gross milk energy content to be about 0.67 kcal/g²⁹⁵ (2.80 kJ/g), but varying from 0.62 to 0.80 kcal/g⁴² (2.59 to 3.35 kJ/g).

1.3 Research questions and aims

1.3.1 Adequacy of exclusive breast-feeding at 6 months of age

The evidence on metabolisable milk intake in exclusively breast-fed infants as reviewed by Reilly and colleagues³, was subsequently compared with the FAO/WHO/UNU reference for mean energy requirements⁴. In brief, milk intake was deemed sufficient to cover energy requirements at 3 - 4 months of age. In more detail, the weighted mean milk intake at 6 months of age (894 ± 87 g/d) and the mean milk energy content of 2.6 kJ/g calculate into a mean TEI of 2324 kJ/d. Corrected for different intakes between boys and girls, the mean TEI were 2439 kJ/d and 2207 kJ/d for boys and girls, respectively⁴. The studies included in the systematic review did not allow a formal weighted analysis of the mean infant weight in those studies.

Instead, the 50th percentile from the UK 1990 reference data for infant weight was used, where median body weights are 8.0 kg and 7.7 kg for boys and girls, respectively, at 6 months of age²⁹⁶. As the FAO/WHO/UNU reference for breast-fed infants are 325 kJ/kg*d and 330 kJ/kg*d for boys and girls at 6 months⁷, respectively, the calculated mean infant energy requirements were 2600 kJ/d for boys and 2541 kJ/d for girls. Hence at 6 months of age, the mean values suggested a shortfall in energy supply of around 6% for boys and 13% for girls during exclusive breast-feeding at 6 months of age.

1.3.2 The hypothesis of the First-Feed study

The lack of evidence of an increase in milk intake over time beyond 3 - 4 months of age³, and the suggested shortfall in energy supply during exclusive breast-feeding at 6 months of age⁴ formed the basis for the research question for the First-Feed study, which was: “To explore how exclusive breast-feeding to 6 months of age is achievable - mainly from an energy balance point of view”. The objective was to use a longitudinal design with measurements of infant milk intake and energy balance, using the DLW method, as well as measurements of growth, to explore changes and adaptations in these variables during exclusive breast-feeding, as defined by WHO², to 6 months of age.

More specifically, the present thesis sought to test the hypotheses derived from Reilly & Wells⁴, where exclusive breast-feeding, as defined by WHO², to 6 months is only adequate if:

- 1) Exclusively breast-fed infants are small in size and/or growing slowly compared to growth references.
- 2) Exclusively breast-fed infants' metabolisable milk intake is higher than literature values.
- 3) Exclusively breast-fed infants have low energy requirements compared to references for infant energy requirements.
- 4) The mothers use strained breast-feeding practices manifest as frequent breast-feeds and/or long durations of breast-feeds in order to provide adequate milk for exclusively breast-fed infants to 6 months of age.

These hypotheses will be further detailed in the introductions of each of the result Chapters 3 - 7. Furthermore, the First-Feed study was used to evaluate the precision of anthropometry measurements as they were performed on the infants (Chapter 3) and the dose-to-infant procedure as part of the DLW method was evaluated (Chapter 4). Finally, the First-Feed study was also used to evaluate the accuracy of prediction equations for infant energy requirements when used on infants exclusively breast-fed as defined by WHO² (Chapter 6).

CHAPTER 2 METHODS

2.1 The First-Feed study

The present chapter documents the general methods used in the First-Feed study. It includes considerations on the study design, an account of ethics approval of the protocol and subsequent amendments, and the funding arrangements. There is a detailed account of the recruitment process and the data-collection including a description of the methods and measurements performed. The methods of infant anthropometric measurements and dose administration¹⁵ will be evaluated in Chapters 3 and 4, respectively. The methods only relevant to each of the result Chapters 3 - 7 are described in further detail in the method sections of these chapters. Finally, the present chapter also describes the general statistical considerations and considerations on power and sample size. Data-sheets and questionnaires used for data collection are included in Appendix A.

2.1.1 Study design

An observational study

As mentioned in section 1.1.3, the best quality evidence is obtained from double-blind randomised controlled trials^{48,49}. Although the recommendation of exclusive breast-feeding to 6 months was not officially adopted by the Scottish Government at the start of the First-Feed study, it would be considered unethical to randomise mothers to exclusively breast-feed for 6 months, or not to, given the current scientific evidence for the possible benefits of exclusive breast-feeding. In addition, mothers may oppose pressures to be told how long to be exclusively breast-feeding for, and would prefer receiving sufficient information and support to be able to make their own informed choices regarding infant feeding²⁹⁷. This could cause a high, and probably biased drop-out rate²⁹⁸. Therefore, an observational study seemed to be the best option of design for the First-Feed study, in spite of the risk of confounding⁵⁵ and unrepresentativity, as described in sections 1.1.3 and 1.1.5.

Participating in the present study was time-consuming for the mothers and involved a lot of commitment from them. Additionally, conversations with mothers who were included in the study could have had a supportive effect and encouraged perseverance with exclusive breast-feeding for longer than had the mother not participated in a study. Therefore, an intervention effect could not be excluded. While this intervention effect would influence the mothers' infant feeding behaviour, the primary outcome variables for the present study, were the physiological adaptations during exclusive breast-feeding to 6 months.

A longitudinal study

As described in section 1.2.4, previous studies on milk intake in exclusively breast-fed infants have mainly used a cross-sectional design. Where a longitudinal design was used, the infants were either not exclusively breast-fed as defined by WHO², or they were exclusively breast-fed to less than 6 months of age. In studies on exclusively breast-fed infants at 6 months of age, evidence of milk intake was obtained by a cross-sectional design and using the test-weighing method. However, a cross-sectional design entails a risk of sample selection bias^{298,299}, where the sample characteristics at different time-points differ in a way that affects the outcome variable.

Therefore, the First-Feed study was designed as a prospective longitudinal (cohort) study. The major strength of such design is the ability to provide evidence on changes in variables over time. However, it takes longer time to conduct than a cross-sectional study, and recruitment may also be more difficult and incur a higher risk of sample selection bias due to the extensive commitment involved from the participants. This might affect the representativeness of the study (the external validity), as only well-supported and very committed mothers would be likely to volunteer for this study.

The evidence behind the Reilly-Wells hypothesis³ indicated that milk intake during exclusive breast-feeding was sufficient at 3 - 4 months, but identified a shortfall in breast milk TEI compared to estimated energy requirements at 6 months of age⁴. Therefore, the First-Feed study was designed with two measurements: 1st time-point around 15 weeks of age (3½ months) and 2nd time-point around 25 weeks of age (6 months).

2.1.2 Ethical approval and funding

The present study was approved in March 2007 by the National Health Service (NHS) Research Ethics Committee of Greater Glasgow Primary Care Division (Ref. 07/S0701/15). This ethics committee was used since all the measurements were performed during home visits to the participants in a way that was comparable to the work done by health visitors in the primary care sector.

In May 2007, a substantial amendment was approved, which involved performing an extra baseline urine sample (section 2.3.3) and measure skinfolds on the mother (section 2.5.3). A minor amendment was approved in November 2007, involving changes of wording in the *Information Sheet and Invitation to Participate* (Appendix A.1).

The First-Feed study was funded by a research grant to the amount of £23,728.75 from the Scottish Government's Health Department, Chief Scientist Office (Ref. no. CZH/4/413). For the first two years, the researcher's Ph.D. was part-time, funded by a studentship from a Danish family charity fund (Blegdalens Erhvervs- og Uddannelsesfond; Eng.: Blegdalen's Business and Educational Fund) to the amount of approximately £18,000, which also covered the annual registration fee to the University of Glasgow. Subsequently, the researcher was awarded a studentship to the amount of £32,000 from the Yorkhill Children's Foundation, which facilitated conversion to full-time for years 2 and 3 and a part-scholarship from the Graduate School of the Medical Faculty to cover registration fees.

2.2 The study plan

2.2.1 Recruitment

Recruitment strategy

Based on statistical power considerations (section 2.7.1), it was decided to recruit 60 mother-infant pairs for the study. Recruitment took place from April 2007 to October 2008. Scottish rates of exclusive breast-feeding in 2008 were 37% at 10 days and 27% at 6 - 8 weeks post-partum, according to CHSP-PS data from ISD Scotland¹⁸⁴ (Table 1.2).

To recruit mothers, the researcher frequently attended NHS infant feeding drop-in sessions and local breast-feeding group meetings. At each clinic or meeting, the researcher targeted potential participants as mothers with exclusively breast-fed infants less than 15 weeks old, who intended to continue exclusively breast-feeding until 6 months of age. The study was explained to them individually and they were given an information sheet (Appendix A.1), which included contact details for the researcher. Initially in the conduct of the study, it was then left to the mothers to contact the researcher, if they had further questions or if they were interested in participating. However, this approach resulted in only very few responses. Therefore, the recruitment strategy was changed to be more pro-active, by taking contact details of the approached mothers (all mothers agreed to provide name and telephone numbers). A follow-up telephone call was made when the infant was 11-13 weeks old. During this call any queries from the mother were addressed, and additional time to consider participation was given, if they had not already decided.

Infant feeding drop-in sessions

In the initial phases of planning the study, the manager of the infant feeding advisors for the Greater Glasgow and Clyde Health Board, Mrs. Linda Wolfson, advised us on possible recruitment strategies based on her knowledge of breast-feeding rates in local areas of Glasgow. The NHS clinics approached initially were the Queen Mother's Hospital, the Southern General Hospital, the Millbrae Antenatal Clinic, the Woodhall Clinic, the Milngavie Clinic, the Maryhill Health Centre, The Gorbals Health Centre and the Annoxtown Initiative Offices. The contacts at the Maryhill Health Centre, the Annoxtown Initiative Offices and the Gorbals Health Centre revealed that it was unlikely that any mothers could be recruited from there. The Milngavie Clinic could not offer the opportunity for recruitment from their clinic initially, but did offer permission to recruit from there at a later stage. The Southern General Hospital had their drop-in session at the same time as the Queen Mother's Hospital, so this clinic was only visited by the researcher once, but posters and information sheets were on display in the clinic. At the Millbrae Antenatal Clinic, the Queen Mother's Hospital and the Woodhall Clinic there was generally a fair number of mothers committed to exclusive breast-feeding, so these clinics were where the main efforts to recruit were focused.

Breast-feeding support groups

The most important voluntary breast-feeding support group was the Glasgow branch of the La Leche League run by Mrs. Susan Miller and Mrs. Elaine Butt. Mrs. Miller was also able to provide us with valuable information in the initial stages of planning the study. The La Leche League held a monthly meeting where mothers could turn up for peer support and advice on breast-feeding issues. In addition to the La Leche League, the National Childbirth Trust (NCT) was contacted. Most of their groups seemed to be NHS-based, but they were able to help in promoting the study, by printing a poster-advert in the local NCT Newsletter. In addition, a few NCT-leaders promoted the study during antenatal classes.

From attending both infant feeding clinics and breast-feeding support groups the anecdotal impressions were greater attendances at the NHS breast-feeding clinics, but there were more mothers with breast-feeding problems and more mothers that were not exclusively breast-feeding. The breast-feeding groups were attended by fewer mothers, but these mothers were very dedicated to exclusive breast-feeding to 6 months and continuing to breast-feed for an extended duration after that.

Posters and internet discussion forums

Breast-feeding and parenting websites were approached as an alternative way to recruit mothers who might not attend breast-feeding support groups or clinics. Some mothers might have chosen to breast-feed exclusively and not experience any significant problems or have any needs for peer support. Discussion topics were posted on two internet sites^{300,301} with information about the study and an invitation to contact the researcher, if they were interested in more information. When recruiting from the internet or in the NCT newsletter, one extra eligibility criterion of living within one hour's drive from Glasgow City had to be added. Finally, a "word of mouth" effect appeared after the initial participants were recruited, and towards the final stages of recruitment, a handful of mothers had approached the researcher after having heard about the study from a friend who had participated or was participating in the study. This meant that the oral information about the study was given over the phone rather than in person, and the information sheet was e-mailed to them.

The majority (39 mothers) were recruited from the NHS infant feeding drop-in sessions, three were recruited from La Leche League (all of those who were eligible), and the rest were recruited through posters, internet discussion forums and via the “word of mouth” effect. This meant that the mothers were selectively recruited on the basis of having an interest in breast-feeding and also seeking environments supportive of breast-feeding.

2.2.2 Selection criteria

Inclusion criteria

Once the mother had expressed willingness to participate in the study, it was confirmed that she and the infant fulfilled the inclusion criteria when being recruited to the study (Appendix A.2). The initial inclusion criteria were the obvious one of exclusive breast-feeding, as defined by WHO², at the time of recruitment into the study as well as an intention to continue exclusive breast-feeding until 6 months post-partum. In addition, the following inclusion criteria were used, mainly to strengthen the internal validity of the study³⁰² by minimising unwanted variability in growth and infant energy requirements in the sample (e.g. infants born small for gestational age, or preterm infants, who often have different growth patterns from term infants^{303,304}).

To be included in the First-Feed study, the infant had to be:

- a singleton
- born at term (≥ 37 weeks of gestation) and with birth weight $> 2500\text{g}$
- generally healthy since birth (free from illness affecting breast-feeding or energy balance)
- less than 15 weeks of age at the time of recruitment

The mother had to:

- speak English
- not have any current or recent involvement in other research studies that potentially could affect the outcome variables
- not have had any serious illness during pregnancy or post-partum

Exclusion criteria

The exclusion criteria were the occurrence of any major illness in mother or infant affecting breast-feeding or energy balance of the infant, and if the mother did not want to continue participation for whatever reason.

2.2.3 Visits and measurements – an overview

As the present study involved many days with measurements, it was decided to conduct the study as a field study with all data collected during home visits. This was deemed the best way to maintain a sufficient number of participants in the study, since going out for frequent appointments at the hospital could be considered a barrier for participation. Appointments for the visits were made during the recruitment phone call, when the mother had decided she wanted to participate and it had been established that she and the infant fulfilled all inclusion criteria. Figure 2.1 presents a timeline for each mother-infant pair in the study.

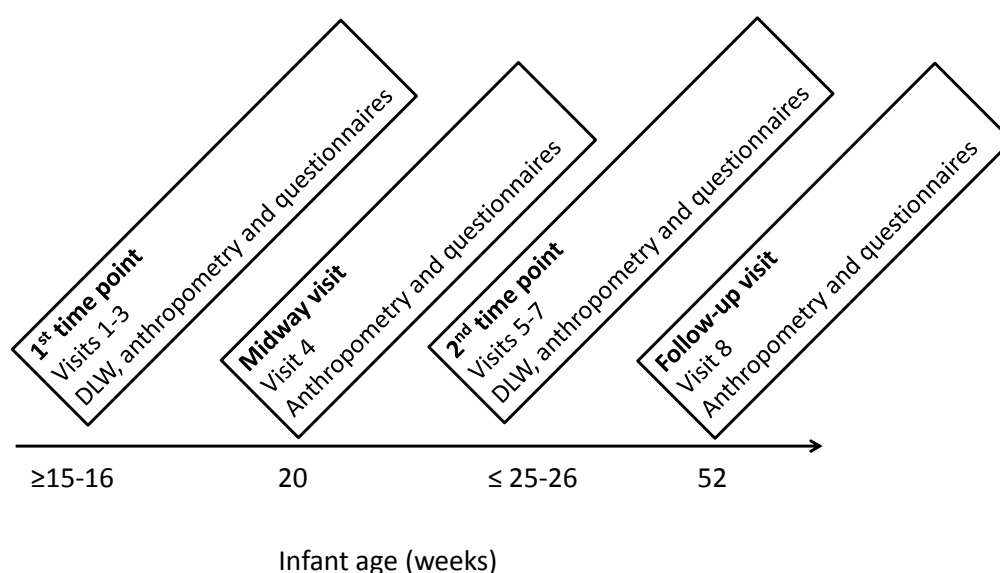


Figure 2.1 Timeline of participation for each mother-infant pair.
DLW: Doubly-labelled water method

Frequency of visits

All home visits (around 370 home visits in total) were conducted by one researcher, the author. There were three visits during each of the 1st and 2nd time-points performed over a period of 8 days for the DLW method (section 2.3). In addition, a mid-way visit and a follow-up visit only included anthropometry and questionnaires.

Given that the First-Feed study was an observational study, there was no guarantee that the mothers managed exclusive breast-feeding to 6 months. Thus the timing of the 2nd time-point had to be designed as reasonably flexible, and the mother was advised to call the researcher if, at any point between the visits, she felt she was struggling with the breast-feeding. If that happened, she would be given the choice of either moving the 2nd time-point forward to be performed earlier while she was still exclusively breast-feeding, or she could introduce complementary foods and would be asked to keep a weighed record of any intake of any complementary foods during the measurement at the 2nd time-point at 25-26 weeks.

Procedures of visits

Figure 2.2 presents an overview of measurements and visits. Each participant had a copy of this flow-chart in her folder to use as a checklist. The first visit the inclusion visit (day -1), where the practicalities of the study were explained in detail to make sure that the mother knew what to expect from participating. This involved repeating the information on visits and measurements and explaining the DLW method to give the mother an understanding of the necessity for careful adherence to the procedures she would be asked to perform. After addressing any queries, written informed consent was obtained (Appendix A.3). Subsequently, measurements of maternal and infant anthropometry were performed and a pre-dose urine sample was taken while giving instructions to the mother for her to perform the subsequent urine sampling (Appendices A.5, A.6, A.8 and A.9). Finally, the mother was given questionnaires to fill in for the next day (Appendices A.4, A.10 and A.11). At visit 2 (day 0), another pre-dose urine sample was taken, the infant was weighed and the DLW was administered (Appendix A.7). For the first 12 mothers recruited to the study, anthropometric measurements which were deemed prone to intra-observer variation were repeated (section 3.2.3).

Figure 2.2 Flow-chart of visits and measurements for the First-Feed study.

Dates																		
Times																		
Visit no.		1	2					3	4	5	6					7		8
Stages	Recruit	1 st time-point							Mid-way	2 nd time-point							Mail	Follow-up
Study day of study week		-1	0	1	2	3-5	6	7	0	-1	0	1	2	3-5	6	7		0
Age of infant (weeks)																		
Information sheet and invitation to participate																		
Recruitment; eligibility, oral information (DS1) ^a																		
Written Informed Consent																		
Background information (DS2) ^a																		
Maternal anthropometry (DS3) ^a																		
Infant anthropometry (DS4) ^a			b								b							
Urine sample from nappy (DS6) ^a																		
Dose to the infant (DS5) ^a																		
Baby Behaviour Diary (BBD)																		
Rothbart's Infant Behavior Questionnaire (IBQ) ^c																		
Breast-feeding practices Questionnaire (BFQ)																		
Complementary Feeding Questionnaire (CFQ) ^c																		

The top two rows were for stating dates and times for appointments. The “white” cells were ticked off when the measurements had been performed or questionnaires had been returned.

^aDS and number refer to number on the Data Sheet – see Appendix A.

^bInfant weight only.

^cThese questionnaires will not be further described in the present thesis as the data has not been used to answer any research aims, but they are included in Appendix A for reference.

On the third visit (day 7) infant anthropometry was measured again and infant urine samples performed by the mother on days 1, 2, 6 and 7 were collected. On some occasions, dose administration was unsuccessful, and if it was possible, another attempt was made on the day after. If the second attempt was unsuccessful as well, or if it was not possible to try the day after for practical reasons, the measurement was abandoned and the third visit was cancelled.

The fourth visit was the mid-way visit at 20 weeks of age, where measurements of infant anthropometry were repeated to obtain an extra data-point of growth, and the mother was given questionnaires to fill in. Visits 5 - 7 constituted the 2nd time-point, where the procedures from visits 1 - 3 were repeated (except informed consent). Finally, a follow-up visit (visit 8) was conducted at 52 weeks of infant age. On this visit, maternal and infant anthropometry was measured and the mother was given the last set of questionnaires to fill in and send back to the researcher in a pre-stamped envelope.

In general, the sequence of measurements at the visits depended very much on the infant's temperament and routines. For instance, if the infant was asleep, time would be spent on measuring maternal anthropometry or filling out questionnaires. If the infant was hungry at the dosing visit, an attempt was made to administer the DLW at the start of the visit, and if the infant had just had a meal, the dose administration would be postponed until the end of the visit. In this way, data collection was balanced with the infant's needs, temperament and willingness to co-operate. Due to the extra time spent on giving information and obtaining consent, this first visit usually lasted about 1½ - 2½ hours. The subsequent visits lasted from ½ - 2 hours.

2.3 The doubly-labelled water method

The main outcome variables were those obtained by the DLW method. Stable isotope methods ($^2\text{H}_2\text{O}$, or $^2\text{H}_2\text{O} - \text{H}_2^{18}\text{O}$) are ideal for measuring infant breast milk intake of free-living exclusively breast-fed infants, since they do not interfere with the breast-feeding routine. As described in the introduction (section 1.2.3), one of the criticisms of the alternative test-weighing method is the way it interferes with breast-feeding, when measurements need to be performed before and after each feed¹³.

2.3.1 Validation studies

Validation of measurements of energy expenditure

The DLW method has been validated in different population groups under various circumstances. Generally, the method is valid for measuring V_{CO_2} with an accuracy of 1 - 3% and a precision of 2 - 8%, when using the multi-point regression analysis³⁰⁵.

Two validation studies using indirect calorimetry (open circuit respiratory gas exchange - see section 1.2.1) as the criterion method in four premature hospitalised infants (2 - 6 weeks old)²⁴⁶, and in nine hospitalised term infants (1 - 2 weeks old) following surgery³⁰⁶ revealed similar results for the measured V_{CO_2} and calculated energy expenditure, when the indirect calorimetry was performed to obtain a representative estimate of TEE. The precision for the DLW method was 4.8%³⁰⁶ and 2.6%²⁴⁶, respectively, for the measurement of TEE. Another study in twelve pre-term infants found an overall agreement of 1% at the group mean, but with more variable results at the individual level³⁰⁷. The DLW method is now considered the criterion method for validation of other methods of measuring components of energy balance^{308,309}.

Validation of milk intake

As described in section 1.2.3, the DLW dose-to-infant method equals the deuterium dose-to-infant method for measuring milk intake. Validation studies in formula-fed infants comparing deuterium dose-to-infant with direct weighing of infant feeding bottles have revealed very similar results, while studies comparing test-weighing with deuterium kinetics generally found lower milk intakes for the test-weighing method.

Outcome variables

Given the convenience of an isotopic method in terms of low burden on the participants, the advantage of obtaining several objective variables from one method (section 1.2.3) and the relatively high precision and accuracy, the DLW method was chosen for the First-Feed study.

In brief, the DLW method produced the outcome variables of TBW and body composition (FFM and FM) based on the principle of dilution, and TEE was derived from isotope elimination rates.

Then estimation of E_{growth} was added to TEE to derive TEI, while variables of water flux from deuterium elimination were used to calculate the amount of metabolisable milk intake. Finally, metabolisable energy and milk intakes were used to calculate the milk energy content (section 2.3.6).

Overall, the DLW method entails five steps: 1) dose administration, 2) urine sampling, 3) urine sample analysis, 4) data-handling to acquire data, and 5) calculations of outcome variables.

2.3.2 Step 1: Dose administration

For the present study, the DLW was prepared from the supplier as sterilised DLW (Rotem Industries Ltd., Beer Sheva, Israel), mixed as 10.40 atom-% H_2^{18}O and >99.9 atom-% $^2\text{H}_2\text{O}$, and the required dose was 2.6 g/kg body weight. The proportion of isotopes as well as the required dose took into account that the dose had to be sufficient to sustain an enrichment at the end of the measurement period that was high relative to the potential analytical error (given the sensitivity of the mass spectrometer)³¹⁰. Furthermore, considerations were given to water turnover in infants relative to the expected energy expenditure²⁴⁸. The dose requirement decided for the present study is very similar to other DLW studies conducted in infants^{242,260,311,312}. The dose requirement per kg body weight was multiplied by a factor of 1.2, to allow for spills of DLW and to have more DLW than required. This factor was decided upon since a previous study using the DLW method had reported that on average 84% of the dose prepared to the infant had been consumed³¹³.

When preparing the dose, a Sartorius Basic (Model BA 310P) precision scale was used with an increment of 1 mg. The scale had been calibrated at the beginning of the study and was not moved during the course of the study. For the procedure of preparing a dose, a data-sheet was used (Appendix A.7), where weights of bottle, dose, dosing equipment and re-sealable bags with tissues to collect spills were recorded both before and after dose administration. The DLW was drawn from the supplied stock (0.5 L bottle) using a 20 ml sterile syringe (VWR Int. Ltd. Lutterworth, UK) and then filtered through a 0.2 micropore filter (VWR Int. Ltd. Lutterworth, UK) into either a 125 ml sterile plastic bottle (Barloworld Scientific Ltd., Staffordshire, UK), or into the infant's own feeding bottle.

In a few cases, the mother had supplied expressed breast milk to mix with the DLW. Then 0.5 g of dose sample was transferred to a 2 ml sample tube (Sarstedt, Leicester, UK), marked study-ID and date, and kept frozen until shipment to University College London for analysis.

Dose administration to the infant

The plan was to administer the DLW to the infant well after the last feed, but before the infant was hungry, which was likely to decrease cooperativeness with dose administration. However, the variability of infants' rhythms precluded a standardised post-prandial time for dose administration. The DLW was administered to the infants undiluted (if not mixed with breast milk) to minimise the amount of water they had to consume. This approach had the disadvantage of any spills contributing more to dose administration error, than if it was diluted. However, it minimised the volume the infant had to consume and thereby decreased the risk of reflux, which was a greater concern in the present study. The dose was administered while the infant was lying in either a supine position on a mat on the floor, or a semi-supine position in a bouncy chair. There were three potential methods of administering the DLW to the infants:

1. Administering the DLW in the infant's own feeding bottle, either alone or mixed with expressed breast milk. This was the preferred method of administration if the infant was familiar with a feeding bottle and was latching on well, but many of the mothers in the present study did not use feeding bottles (Chapter 4).
2. Administering the DLW through a 5 ml syringe. This seemed like the best approach for infants not familiar with a bottle. This approach has also been reported before^{14,260,314}.
3. Administering the DLW through a naso-gastric tube (feeding tube), also described elsewhere^{312,313,315}. In the present study, the feeding tube was attached to the mother's breast with the end of the tube at the nipple to administer the DLW during a feed.

After dose administration, dosing equipment were weighed again to determine the dose consumed. Spills on the tissues were composed of DLW, saliva and sometimes reflux, which was corrected for. The method of dose-to-infant administration of DLW will be evaluated in Chapter 4.

2.3.3 Step 2: Urine sampling

Procedure of urine sampling

The urine sampling procedure has been described and evaluated before^{246,316,317}. Roberts and Lucas evaluated this method for urine collection in infants; they found an evaporation rate of less than 0.4% per hour³¹⁶. In the present study, small pieces of cotton wool were placed “strategically positioned” in a dry nappy intended to be small for the infant to keep it in place. The nappy was checked for urination at least every 30 minutes, but more often as convenient, and voiding time was calculated as the mid-point between the last time it was dry, and the time it was wet (Appendix A.8). The wet cotton wool was placed in a 20 ml syringe (VWR Int. Ltd. Lutterworth, UK) and the plunger was re-inserted to squeeze the urine into a 5 ml plastic sample tube with a screw cap (Teklab Ltd., Durham, UK). If there was not enough for a sample (2.5 ml), the mother was asked to repeat the procedure (using a separate urine sample kit). Extra undated kits were supplied for this purpose. The mother was instructed to keep the urine samples in the freezer until the next visit.

Timing of urine samples

Two pre-dose urine samples were obtained before dose administration to measure natural abundance (background) levels of deuterium and oxygen-18³¹⁸. The choice of two pre-dose samples was decided upon due to the difficulty of measuring the low natural abundance levels of isotopes. A mean of the two samples was used for the calculations to reduce the effect of variation in natural abundance levels^{306,318}.

The first pre-dose urine sample was obtained on the first visit of each week of measurement. During the first visit (visit 1), the procedure was carefully explained to the mother for her to take the subsequent samples. The mother was also given written instructions on how to obtain the urine sample, and was encouraged to contact the researcher in case of any problems (Appendix A.9). It was emphasised to the mother to try and collect the urine samples on approximately the same time of day on the following days (usually in the morning, but not the first voiding) to avoid diurnal variation in hydration levels and rates of energy expenditure. Such variation could affect internal precision of the slope of the isotope elimination lines.

A second pre-dose sample was collected the next day before the dose administration. Subsequently, urine samples were obtained per protocol on days 1, 2, 6 and 7. Using an elimination period of 6 - 8 days has previously shown to result in the best precision^{306,319}. The reliability of the method is improved if the measurement period includes at least 1.5 biological half-life of deuterium³²⁰, where the difference in changes of enrichments of oxygen-18 and deuterium becomes clearly distinguishable, given their different elimination rates. The number of urine samples was a compromise between daily samples, which would be an extra burden on the mother and an extra cost of analysis, and only two (start and end-point) samples, where a great reliance is placed on these samples in terms of accuracy and precision for calculating isotope elimination rates.

Urine sample storage and transport

The mother had kept the urine samples frozen until they were collected at the last visit of each week of measurements. From the mother's home and to the office, the urine samples were transported in a fridge in the car. Upon arrival at the office, they were transferred to a domestic freezer and stored at approximately -20°C. Finally, they were shipped in batches to University College London for mass spectrometry analysis. During shipment the urine samples were in a polystyrene box surrounded by ice packs.

2.3.4 Step 3: Urine sample analysis

Principle of mass spectrometry analysis

The principle of the procedure of Isotope Ratio Mass Spectrometry (IRMS) is illustrated in Figure 2.3. Briefly, the urine sample is vaporised by being flushed with Helium gas. The vaporised sample is ionised when passing through a high intensity beam of electrons, before being accelerated through a curved tube in an electromagnetic field. The molecules will then be deflected on the other side of the magnetic field at different angles depending on their molecular weight, so that counters at the end of the tube can detect the molecules of a particular weight and give a measure of the ratio between the isotopes of the sample. Data on isotopic enrichment of both pre- and post-dose urine samples and dose samples obtained from the mass spectrometer were expressed as a ratio of $^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$, respectively, and expressed as relative delta (δ) per mil units (‰), i.e. relative to Vienna Standard Mean Ocean Water³⁰⁵ (Appendix B).

Logistics of mass spectrometers

At University College London, the analysis was run in batches of up to 88 vials at a time (0.5 ml urine sample in each vial) with a laboratory working standard placed in the middle as well as at the beginning and end of each batch.

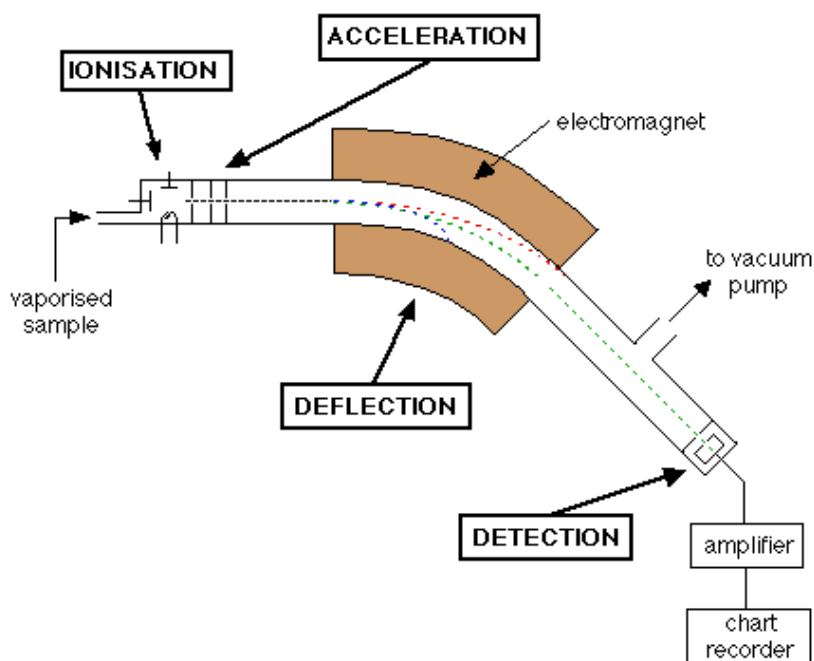


Figure 2.3 A diagram of the principle of Isotope Ratio Mass Spectrometry³²¹.

For the measurement of the ratio of $^{18}\text{O}/^{16}\text{O}$ (hereafter oxygen-18), each vial was flushed with 0.3% CO_2 in Helium gas and equilibrated at room temperature for 24 hours before analysis³²². For the measurement of the ratio of $^2\text{H}/^1\text{H}$ (hereafter deuterium), each vial was flushed with 2% H_2 gas and allowed equilibrium catalysed by a platinum rod for >12 hours at room temperature before analysis³²³. Each sample was analysed twice for both deuterium and oxygen-18, and values for calculations were based on mean values, except in a small number of cases where there was not enough urine for duplicate analysis.

The first ten paired samples were analysed for both deuterium and oxygen-18 on a ThermoElectron delta⁺ XP (Bremen, Germany) at University College London in August 2008. After this time, problems with this mass spectrometer meant that deuterium analysis was precluded. Therefore, the remainder of the samples were analysed for deuterium on a Europa Scientific Ltd. (Crewe, UK) mass spectrometer at IsoAnalytical (Crewe, UK) in a similar way.

Instrument precision

Instrument precision was assessed for the two mass spectrometers using the method by Bland and Altman for calculating repeatability coefficient³²⁴. The SD's of the differences between the duplicate (paired) analyses were calculated as³²⁴:

$$SD = \sqrt{\frac{\sum(pre_1 - pre_2)^2}{n}}$$

The coefficient of repeatability is defined as 2SD - equivalent to the range which will include 95% of the differences between duplicate analyses³²⁴. The duplicate analyses of the pre-dose samples for deuterium were used, since the natural abundance levels were the lowest and therefore most difficult to measure. Furthermore, the natural abundance levels for deuterium were measured on both mass spectrometers for some samples and could therefore work as a comparison.

For the pre-dose urine samples analysed for deuterium at University College London, the mean \pm SD difference between the duplicate analyses (both day -1 and day 0) was $0.49 \pm 1.89\%$ and the repeatability coefficient was 3.78% ($n = 24$). Likewise, for the pre-dose urine samples analysed for deuterium at IsoAnalytical there was a mean \pm SD difference between the duplicate analyses was $-0.01 \pm 1.57\%$ and the repeatability coefficient was 3.14% ($n = 120$). Elsewhere, the instrument precision of measuring natural abundance levels has been found to be 0.1% for deuterium and 0.3% for oxygen-18³²⁵. However, the values of instrument precision obtained in the present study is $<1\%$ of the enrichment levels expected after dose administration.

2.3.5 Step 4: Data-handling

The data-handling process consisted of creating individual elimination spreadsheets based on isotope data for each measurement using a pre-coded spreadsheet developed by Dr. W.A. Coward¹⁴. These were then checked against original data, as specified below. Subsequently, the elimination spreadsheets were used to evaluate the quality of each dose administration and analysis according to criteria described below.

Original data sets

Enrichment data from the mass spectrometer were transferred to data spreadsheets (by staff operating mass spectrometers), and these were used as original data. Other original data were weights of the infants, urine sampling times, and the amount of dose consumed. The weights from day -1, or day 0, and day 7 were used as basis for the calculations. Post-dose urine sampling times were expressed in days with three decimal places. For instance, for a urine sample taken 23 hours after the time of dose administration (day 1), this would be 0.958 days. Finally, the dose consumed was first calculated as the dose available to the infant (from pre- and post- weighing the dosing equipment) minus the spills that had been caught on tissues. However, given that the spills were unlikely to be consumed only of dose this method would introduce a (systematic) bias in the dose consumed. Therefore, the comments made at the dosing visits were used to calculate a correction factor. For instance, if the spills caught on tissues were observed as mixed with saliva (higher viscosity) or breast milk residues, a correction was made, assuming half of the spills was saliva or milk and half was dose¹⁵. By this principle, some amounts of dose may be overestimated, while others may be underestimated, but this was expected to even out, when results were regarded at the group level. For further details on the evaluation of dose administration, see Chapter 4.

Creation and check of isotope elimination spreadsheets

The original data on isotope enrichments, decimal times for urine sampling, infant weights and the corrected dose consumed were manually copied into pre-coded spreadsheets by staff at University College London. One elimination spreadsheet was created for each measurement. All the equations for calculating the elimination rates and other outcome variables are freely available, but the design of the pre-coded spreadsheet, which was developed specifically for the DLW method by W.A. Coward¹⁴, is the intellectual property of the Medical Research Council (Cambridge, UK). Therefore, the handling of elimination spreadsheets happened in collaboration with staff at University College London (through e-mail correspondence and at meetings) over the period of September 2009 to February 2010.

Quality criteria for isotope analysis

Once checked and corrected, the elimination spreadsheets were evaluated individually for quality. First, the space ratio (Appendix B.12) was used as an indicator of analytical error. The typical space ratio is 1.034^{326,327} because more deuterium (about 4%) than oxygen-18 (about 1%)^{328,329} leaves the water pool and exchanges with non-aqueous molecules, and thus the dilution space of deuterium is bigger than the dilution space of oxygen-18^{305,327}. In the present study, the measurement was therefore rejected due to analytical error if the space ratio was found to be either <1.000 or >1.090.

The elimination spreadsheet calculated an overall error term, which included both analytical error, error caused by the sampling procedure and biological variation³¹⁹. It was decided to use a cut-off on the error of ≤10% as quality criteria of the measurement.

The elimination spreadsheet also produced residual plots as part of the regression to produce the best fit elimination curve of each isotope. The residual plots were explored for covariance between isotopes in order to identify any non-random errors, and if this was the case, the data from individual days could be deleted from the spreadsheet. If two days were deleted the method was essentially reduced to the two-point (plateau) method (this applied to four measurements).

Principles of calculations in the pre-coded spreadsheet

The pre-coded spreadsheet¹⁴ was designed to plot a decay curve (Figure 2.4) of the natural logarithm of oxygen-18 and deuterium enrichment in body water, which was extrapolated back to zero time (the back-extrapolation method)²⁵⁰. The dilution space of deuterium and oxygen-18, N_D and N_O , was calculated as:

$$N_{O \text{ or } D} = \frac{AT}{a} \times \frac{(E_d - E_t)}{(E_s - E_p)}$$

where A is the dose given, T is the volume of tap water in which the dose is diluted, a is the portion of dose diluted, E is the enrichment of the following; d - dose, t - tap water, s - post dose and p - pre-dose²⁵⁰ (Appendix B.2).

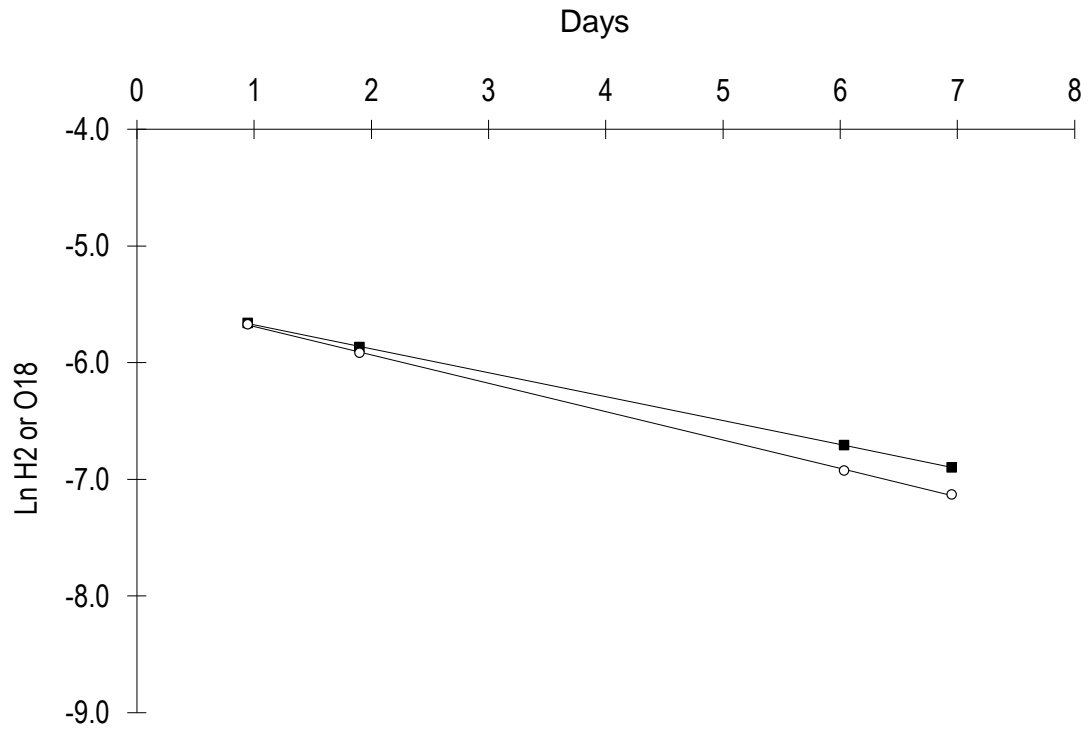


Figure 2.4 Example of a decay curve with the natural logarithm of the isotope enrichment against time. Black squares represent deuterium, circles represent oxygen-18.

TBW was calculated as an average of the dilution spaces of deuterium and oxygen-18 after correction for non-aqueous exchange using the factors of 1.01 and 1.04 for oxygen-18 and deuterium, respectively^{329,330}.

V_{CO_2} was calculated as^{255,331}:

$$V_{CO_2} = \frac{(N_O \times k_O)}{2 \times f_3} - \frac{(N_D \times k_D) \times [(x \times f_2) + 1 - x]}{2 \times f_3 \times [(x \times f_1) + 1 - x]}$$

where k_O and k_D are the oxygen-18 and deuterium elimination rate constants respectively, f_1 is the fractionation factor for deuterium between vapour and liquid (0.93)³³², f_2 is the fractionation factor for oxygen-18 between vapour and liquid (0.99), f_3 is the fractionation factor for oxygen-18 between CO_2 and water (1.04), and x is the proportion of water fractionated (assumed to be 0.15)³³³. Oxygen consumption was predicted from V_{CO_2} using a RQ of 0.85 to facilitate comparability with other studies³³⁴ (section 1.2.1 and Appendix B.5). Finally, TEE was calculated using Weir's equation²⁴³.

2.3.6 Step 5: Calculation of outcome variables

From the elimination spreadsheets, variables were copied into one separate spreadsheet for all the infants with a successful measurement. Table 2.1 is a list of the variables transferred into this spreadsheet.

Table 2.1 List of variables from original data sets as they were transferred to the spreadsheet for calculation of outcome variables.

Column	Variable name	Variable label or formula for calculation ^a
A	Study ID	Date of birth and initials
B	Sex	girl = 1, boy = 0
C	Age1_day0	Age visit 2 (day 0) (d)
D	Age1_d02	Age1_day0 * Age1_day0 ^a
E	Age1_day7	Age visit 3 (day 7) (d)
F	Age1_d72	Age1_day7 * Age1_day7 ^a
G	Age2_day0	Age visit 6 (day 0) (d)
H	Age2_day02	Age2_day0 * Age2_day0 ^a
I	Age2_day7	Age visit 7 (day 7) (d)
J	Age2_day72	Age2_day7 * Age2_day7 ^a
ANTHROPOMETRY		
K	Weight_Age1_d0	Weight Age 1 day 0 (g)
L	Weight_Age2_d0	Weight Age 2 day 0 (g)
M	Weight_Age1_d7	Weight Age 1 day 7 (g)
N	Weight_Age2_d7	Weight Age 2 day 7 (g)
O	dWeight1	Change in Weight d7 - d0, Age 1 (g) ^a
P	dWeight2	Change in Weight d7 - d0, Age 2 (g) ^a
MASS SPEC DATA		
Q	Error1	mass spec error Age 1
R	Error2	mass spec error Age 2
S	Space_ratio1	Space ratio Age 1
T	Space_ratio2	Space ratio Age 2
U	TEE1	TEE Age 1 (kJ/d)
V	TEE2	TEE Age 2 (kJ/d)
W	FM1	Fat mass Age 1 (g)
X	FM2	Fat mass Age 2 (g)
Y	Fat_percent1	Fat % Age 1
Z	Fat_percent2	Fat % Age 2
AA	O18Space1	O18 dilution space Age 1 (mol)
AB	O18space2	O18 dilution space Age 2 (mol)
AC	D2Space1	D2 dilution space Age 1 (mol)
AD	D2Space2	D2 dilution space Age 2 (mol)
AE	kO1	elimination constant O18 Age 1 (/d)
AF	kD1	elimination constant D2 Age 1 (/d)
AG	kO2	elimination constant O18 Age 2 (/d)
AH	kD2	elimination constant D2 Age 2 (/d)
AI	TBW1	TBW Age 1 (g)
AJ	TBW2	TBW Age 2 (g)
AK	FFM1	Fat free mass Age 1 (g)
AL	FFM2	Fat free mass Age 2 (g)

^a Variables that were calculated.

Age1: 1st time-point, Age2: 2nd time-point, TEE: Total Energy Expenditure, FM: Fat Mass, k: Elimination rate constant, TBW: Total Body Water, FFM: Fat Free Mass

Subsequently, outcome variables were calculated using the approach developed by Lucas and colleagues²⁷⁹ and refined by Davies and Wells^{250,255}. Table 2.2 is a list of all the calculations in the sequence they were performed.

Energy deposited in growth

E_{growth} (kJ/d) was calculated from weight gain over the 7 days of the TEE measurement, as previously described elsewhere²⁵⁵. FFM on day 0 was calculated using an age- and sex-specific factor of hydration of FFM, which was derived from regression equations on data of the reference child²⁴⁹. FM on day 0 was then calculated as body weight minus FFM. Weight gain over the 7 days was assumed to have a linear increase in the proportions of FM and FFM. Gain in FM (g) was calculated directly as the difference in FM between day 0 and day 7. Gain in FFM (g) was corrected for age- and sex- specific changes in hydration and in the proportion of FFM that is protein, using regression equations derived from data on the reference child²⁴⁹, resulting in a value of protein gain. Finally, the energy stored as fat and protein was calculated, assuming a standard caloric value of 38.7 kJ/g for fat and 23.6 kJ/g for protein³³⁵ and summarised to produce a value for E_{growth} (kJ/d). The standard calorific values were used rather than specific calorie conversion factors obtained for breast milk (fat: 36.87 kJ/g, protein: 19.99 kJ/g)³³⁶ in order to facilitate comparison with other studies.

Total metabolisable energy intake

Daily TEI was calculated as the daily average TEE (kJ/d) plus the daily average E_{growth} (kJ/d), as described by Lucas and colleagues²⁷⁹.

Metabolisable breast milk intake

The elimination rate of deuterium was used to calculate amount of daily milk intake (g/d)²⁷³ and correcting for environmental water influx²⁴⁴:

$$\text{Milk intake (g/d)} = 0.88 \frac{\left(\frac{N_D \times k_D}{f_1} \right)}{0.96}$$

Metabolisable milk energy content

Milk energy content (kJ/g) was calculated as TEI (kJ/d) divided by milk intake (g/d)²⁷⁹.

Table 2.2 List of calculated variables for metabolisable milk intake, E_{growth} , metabolisable energy intake and milk energy content.

Column	Variable name	Formula for calculation
AM	Water_efflux_1	$D2\text{Space1} * kD1 * 18.02/0.99 \text{ (g/d)}$
AN	Water_efflux_2	$D2\text{Space2} * kD2 * 18.02/0.99 \text{ (g/d)}$
AO	Water_stored_1	$d\text{Weight1 (g)} * \text{TBW1 (g)} / \text{Weight_Age1_d0 (g)} / 7\text{d (g/d)}$
AP	Water_stored_2	$d\text{Weight2 (g)} * \text{TBW2 (g)} / \text{Weight_Age2_d0 (g)} / 7\text{d (g/d)}$
AQ	Water_influx_1	$\text{Water_efflux_1} + \text{water stored_1 (g/d)}$
AR	Water_influx_2	$\text{Water_efflux_2} + \text{water stored_2 (g/d)}$
AS	Milk_water_1	$0.937 * \text{Water influx_1 (g/d)}$
AT	Milk_water_2	$0.937 * \text{Water influx_2 (g/d)}$
AU	Milk_intake_1	$\text{Milk water_1} / 0.96 \text{ (g/d)}$
AV	Milk_intake_2	$\text{Milk water_2} / 0.96 \text{ (g/d)}$
BODY COMPOSITION DATA		
AW	TBW_Age1_d0	$O18\text{space1} * 18.02 / 1.01 \text{ (g)}$
AX	TBW_Age2_d0	$O18\text{space2} * 18.02 / 1.01 \text{ (g)}$
AY	Age_sex_TBW_frac_FFM_Age1_d0	Age-sex-specific regression of TBW fraction of FFM Age1_d0
AZ	Age_sex_TBW_frac_FFM_Age2_d0	Age-sex-specific regression of TBW fraction of FFM Age2_d0
BA	FFM_Age1_d0	$\text{TBWAge1_d0} / \text{Age-sex TBW fraction FFM from regression (g)}$
BB	FFM_Age2_d0	$\text{TBWAge2_d0} / \text{Age-sex TBW fraction FFM from regression (g)}$
BC	FM_Age1_d0	$\text{Weight_Age1_d0} - \text{FFM_Age1_d0 (g)}$
BD	FM_Age2_d0	$\text{Weight_Age2_d0} - \text{FFM_Age2_d0 (g)}$
BE	Age_sex_protein_frac_FFM_Age1_d0	Age-sex-specific regression of protein fraction of FFM_Age1_d0
BF	Age_sex_protein_frac_FFM_Age2_d0	Age-sex-specific regression of protein fraction of FFM_Age2_d0
BG	protein_mass_Age1_d0	$\text{FFM_Age1_d0} * \text{Age_sex_protein_frac_FFM_Age1_d0 (g)}$
BH	protein_mass_Age2_d0	$\text{FFM_Age2_d0} * \text{Age_sex_protein_frac_FFM_Age2_d0 (g)}$

Age1: 1st time-point

Age2: 2nd time-point

TEE: Total Energy Expenditure

FM: Fat Mass

k: Elimination rate constant

TBW: Total Body Water

FFM: Fat Free Mass

Table 2.2 continued List of calculated variables for metabolisable milk intake, E_{growth} , metabolisable energy intake and milk energy content.

Column	Variable name	Formula for calculation
BI	Age_sex_protein_frac_FFM_Age1_d0	Age-sex-specific regression of protein fraction of FFM_Age1_d0
BJ	Age_sex_protein_frac_FFM_Age2_d0	Age-sex-specific regression of protein fraction of FFM_Age2_d0
BK	protein_mass_Age1_d0	FFM_Age1_d0 * Age_sex_protein_frac_FFM_Age1_d0 (g)
BL	protein_mass_Age2_d0	FFM_Age2_d0 * Age_sex_protein_frac_FFM_Age2_d0 (g)
BM	TBW_frac_BW_Age1_d0	TBW_Age1_d0 (g) / Weight_Age1_d0 (g)
BN	TBW_frac_BW_Age2_d0	TBW_Age2_d0 (g) / Weight_Age2_d0 (g)
BO	Age_sex_TBW_frac_BW_Age1_d0	Age-Sex-specific regression TBW fraction of Weight_Age1_d0
BP	Age_sex_TBW_frac_BW_Age1_d7	Age-Sex-specific regression TBW fraction of Weight_Age1_d7
BQ	Age_sex_TBW_frac_BW_Age2_d0	Age-Sex-specific regression TBW fraction of Weight_Age2_d0
BR	Age_sex_TBW_frac_BW_Age2_d7	Age-Sex-specific regression TBW fraction of Weight_Age2_d7
BS	regression_proportion_Age1	proportion TWB_frac_Weight_Age1 d7 / d0
BT	regression_proportion_Age2	proportion TWB_frac_Weight_Age2 d7 / d0
BU	TBW_Age1_d7	Weight_Age1_d7 * TBW_frac_Weight_Age1_d0 * regression_proportion_Age1 (g)
BV	TBW_Age2_d7	Weight_Age2_d7 * TBW_frac_Weight_Age2_d0 * regression_proportion_Age2 (g)
BW	Age_sex_TBW_frac_FFM_Age1_d7	Age-sex-specific regression of TBW fraction of FFM Age1_d7
BX	Age_sex_TBW_frac_FFM_Age2_d7	Age-sex-specific regression of TBW fraction of FFM Age2_d7
BY	FFM_Age1_d7	TBW_Age1_d7 / Age-sex-TBW_frac_FFM_Age1_d7 (g)
BZ	FFM_Age2_d7	TBW_Age2_d7 / Age-sex-TBW_frac_FFM_Age2_d7 (g)
CA	FM_Age1_d7	Weight_Age1_d7 - FFM_Age1_d7 (g)
CB	FM_Age2_d7	Weight_Age2_d7 - FFM_Age2_d7 (g)
CC	Age_sex_protein_frac_FFM_Age1_d7	Age-sex-specific regression of protein fraction of FFM_Age1_d7
CD	Age_sex_protein_frac_FFM_Age2_d7	Age-sex-specific regression of protein fraction of FFM_Age2_d7
CE	protein_mass_Age1_d7	FFM_Age1_d7 * Age-sex-specific protein fraction of FFM_Age1_d7 (g)
CF	protein_mass_Age2_d7	FFM_Age2_d7 * Age-sex-specific protein fraction of FFM_Age2_d7 (g)

Age1: 1st time-pointAge2: 2nd time-point

TEE: Total Energy Expenditure

FM: Fat Mass

k: Elimination rate constant

TBW: Total Body Water, FFM: Fat Free Mass

Table 2.2 continued List of calculated variables for metabolisable milk intake, E_{growth} , metabolisable energy intake and milk energy content.

Column	Variable name	Formula for calculation
CG	dFM_Age1	FM_Age1_d7 - FM_Age1_d0 (g)
CH	dFM_Age2	FM_Age2_d7 - FM_Age2_d0 (g)
CI	dprotein_mass_Age1	Protein_mass_Age1_d7 - Protein_mass_Age1_d0 (g)
CJ	dprotein_mass_Age2	Protein_mass_Age2_d7 - Protein_mass_Age2_d0 (g)
ENERGY DATA		
CK	Estored_Age1	dFM_Age1 (g) * 38.7 (kJ/g) + dprotein_Mass_Age1 (g) * 23.6 (kJ/g) (kJ)
CL	Estored_Age2	dFM_Age2 (g) * 38.7 8kJ/g) + dprotein_Mass_Age2 (g) * 23.6 (kJ/g) (kJ)
CM	Estored_Age1_kJ_d	Estored_Age1 / 7 days (kJ/d)
CN	Estored_Age2_kJ_d	Estored_Age2 / 7 days (kJ/d)
CO	TEI_Age1	Estored_Age1_kJ_d + TEE1 (kJ/d)
CP	TEI_Age2	Estored_Age2_kJ_d + TEE2 (kJ/d)
CQ	Emilk_Age1	TEI_Age1 (kJ/d) / milk_intake_Age1 (g/d) (kJ/g)
CP	Emilk_Age2	TEI_Age2 (kJ/d) / milk_intake_Age2 (g/d) (kJ/g)

Age1: 1st time-point

Age2: 2nd time-point

TEE: Total Energy Expenditure

FM: Fat Mass

k: Elimination rate constant

TBW: Total Body Water

FFM: Fat Free Mass

2.4 Infant anthropometry

Purpose and choice of measurements

In order to evaluate infant growth during the study, it was necessary to obtain comprehensive data on infant anthropometry. To ascertain normal growth, selected anthropometric variables were expressed as z-scores relative to the WHO Child Growth Standards (Chapter 3). The choice of methods of measurements was limited to those methods which were suitable to use in the field³³⁷ and it was an advantage to use methods comparable to those used by the WHO for the Multicentre Growth Reference Study (MGRS)³³⁸ (section 3.1.3). Several methodologies for measuring body composition, including densitometry and elemental analysis³⁰⁵ are not feasible for a field setting and it was not within our means to use the method of air-plethysmography (PEA POD®)³³⁹. In addition, head circumference, stomach circumference and mid-upper-arm circumference were excluded due to high imprecision compared to sensitivity of the method³³⁷ (i.e. to detect a change within the duration of the study, a much larger sample size would be needed). Finally, the number of measurements performed had to be balanced between the benefit of obtaining more data to explore and the risk of fatigue affecting the quality of the measurements. The measurements chosen included crown-heel-length, body weight as well as triceps and subscapular skinfolds, which were performed comparatively to the MGRS³³⁸. Knee-heel-length was included as an extra measurement of linear growth. The measurements were recorded on data-sheet 4 (DS4, Appendix A.6).

2.4.1 Body weight

Body weight was measured, while the infant was nude, on an electronic infant weighing scale (SECA 835, Numed, Sheffield, UK) with an increment of 20 g. A towel was placed on the scale which was reset to zero before each reading. Two readings were averaged to produce one measurement, except on a few occasions where the infant was too unco-operative to obtain a second reading.

2.4.2 Crown-heel-length

Crown-heel-length was measured to the last completed 0.1 cm on a Kiddimetre (Raven Equipment, Castlemead, UK).

The infant was positioned with the head towards the fixed vertical end-plate of the measuring board (Figure 2.5). To achieve a vertical Frankfort Plane with the apex of the head touching the end-plate, the mother was asked to sit by the head end of the measuring board, hold the infant's head towards the end-plate, and try to get the infant to look up at her. The investigator then kept the infant's legs stretched by pushing down on the knees. The reading was taken when the mobile end-plate touched the infants' heels. Three readings were averaged to produce one measurement.

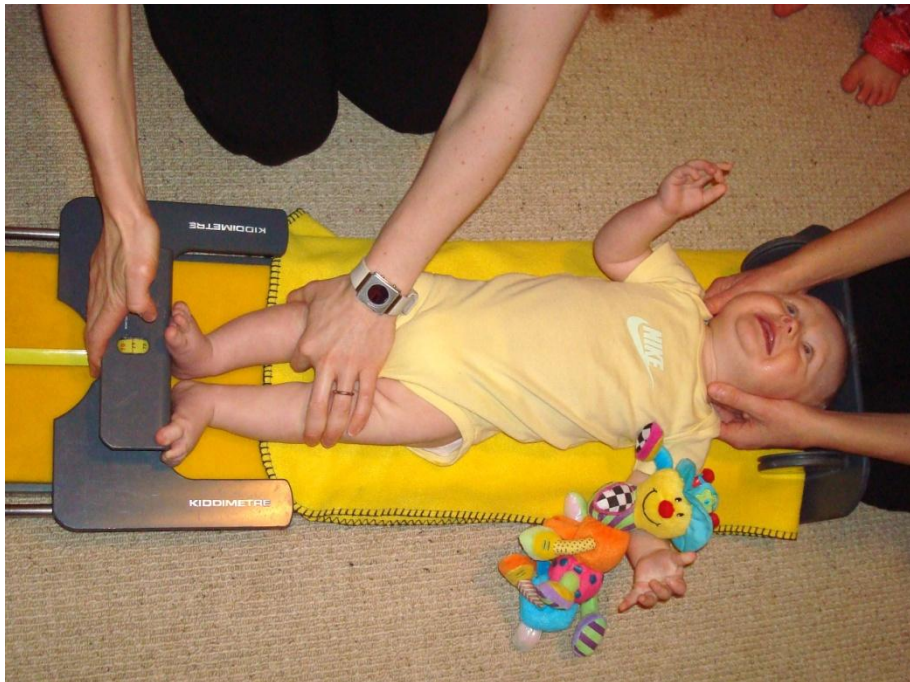


Figure 2.5 Photograph of measurement of supine length on a measuring board.

2.4.3 Knee-heel-length

The purpose of the knee-heel-length measurement was to obtain an additional measurement of linear growth, using a hand-held infant knemometer (FORCE Technology, Denmark) with an increment 0.01 mm. A knemometer resembles a pair of electronic callipers (Figure 2.6). The arm with the knee bracket is fixed on a metal plate, which is attached to a ruler. The arm with the heel bracket moves along the ruler, and is connected to an electronic display measuring the distance between the end of the metal plate and the heel bracket.

The infant was positioned on its back with the right leg flexed to an angle of 90° at both hip and knee joints³⁴⁰.

The knemometer was placed with knee and heel in the corresponding brackets and the knemometer made an electronic reading of the distance from the heel bracket to the metal plate, when the pressure applied to the heel bracket reached a preset value. The reading was automatically transferred to a printer connected to the display. An average of five sequential readings added to the length of the metal plate (constant) constituted one measurement.



Figure 2.6 Positioning of a knemometer for the measurement of knee-heel-length.

Accuracy and precision of the hand-held knemometer

The hand-held infant knemometer has been validated in pre-term infants with a total error of 0.82 mm^{340} . For comparison, the knee-heel length growth velocity is approximately 0.4 mm/d in newborn infants³⁴¹. Overall, the knee-heel-length is considered to be an accurate and precise measure of linear growth velocity in infants. Its advantages are the measurement of the lower leg only, thereby avoiding several flexible joints that increase inaccuracy and imprecision of a measurement^{340,342}, and the fact that less co-operation from the infant is required than for the crown-heel-length measurement. On the other hand, precision of the knee-heel-length measurement can in theory be affected by day-to-day variation in hydration levels in the soft tissue covering the knee and the heel³⁴⁰, but a commentary on this found fluctuations in tissue hydration not to be an issue³⁴¹.

In general, day-to-day variation and within-day variation is probably smaller for knee-heel-length than for crown-heel-length measurements³⁴². Precision can be reduced due to positioning of the knemometer, where the subtle changes in the angling of the knee and angle joints can influence the reading³⁴⁰. In the present study, the higher muscle tone of mature active infants, compared to the premature infants used in validation studies, could compromise the positioning of the knemometer through all five readings. One longitudinal study found lower precision of the knee-heel-length measurement when infants were older³⁴³. Therefore the error of the measurement as conducted in the present study is unlikely to be as low as in validation studies^{340,343}.

Because of its relatively high precision, the knee-heel-length is very suitable for evaluation of short-term linear growth velocity. However, as the body changes proportions during growth and the proportion of crown-heel-length/height that is the lower leg increases with age^{341,342}, the growth velocity of the lower leg does not correlate linearly with crown-heel-length growth velocity and can therefore not be used as a predictor of crown-heel-length³⁴³. Also, growth has been proposed to happen in mini-spurts or saltations^{344,345}, and although this theory is controversial, it has great impact on the use of knee-heel-length for the evaluation of growth velocity at the individual level, because of the timing of these changes in growth relative to the measurements³⁴².

2.4.4 Skinfold thickness

Skinfold thickness measurements are considered indices of body composition³⁴⁶ and provide measurements of subcutaneous fat. In the present study, skinfold thickness was measured using a Holtain Caliper (Holtain, Crymych, UK) to the last completed 0.2 mm on the left side of the infant. Subscapular and triceps skinfolds represented truncal and limb subcutaneous fat, respectively³⁴⁷.

The skinfolds were measured with the infant sitting on the mother's lap or resting up against her shoulder. For subscapular skinfolds, the inferior angle of the scapula was palpated and a skinfold was pinched at an angle of approximately 45°. For the triceps skinfold, a measuring tape was used to identify the halfway point between the acromion process and the tip of the olecranon process. A little dot was made with a pen in order to be able to identify the same spot between readings. Then a vertical skinfold was pinched.

The skinfold calliper exerts a constant pressure ($10\text{g}/\text{mm}^2$) on the skinfold, and each skinfold pinch was not held for more than 1 - 2 seconds to avoid excess compression of the skinfold. For each skinfold measurement, three readings were made to produce one averaged measurement, except in a few cases where the infant got upset and the measurement was then recorded as an average of two readings.

2.5 Maternal anthropometry and body composition

The measurements of maternal anthropometry were mainly used to characterise the mothers at a group level. The measurements were recorded on data-sheet 3 (Appendix A.5).

2.5.1 Height, weight and body mass index

Height was measured using a portable stadiometer; Leicester Height Measure (Child Growth Foundation, London, UK), which was placed against a wall. The mother was positioned with her back side against the stadiometer and her head held with the Frankfort Plane horizontal. The mother was asked to make a maximal inhalation and hold her breath for the duration of each reading. The measurement was performed as three readings to the last completed 0.1 cm and an average value was used for calculation of BMI ($\text{weight (kg)}/\text{height}^2 (\text{m}^2)$).

Weight was measured as part of the body composition measurement described below to the nearest 0.1 kg, wearing light clothes and no shoes or socks. Only one reading of weight was performed, and this was used to calculate BMI.

2.5.2 Body composition

Weight and body composition of mothers were measured on a Tanita® Body Composition Analyser (Model TBF-300; Middlesex, UK) using bioelectrical impedance. This method uses a weak electrical current ($800 \mu\text{m}$; 50KHz) sent through the legs and lower trunk from source (anterior) to sink (posterior) electrodes on a metal sole-plate. The current can easily pass through FFM (electrolyte-containing tissue) but meets resistance from fat tissue. The resistance to the current of the legs and lower trunk results in a voltage drop, which is measured as impedance (Ohms), used to calculate body composition.

A value of 0.5 kg for clothing was used to reduce systematic bias (over-estimation of body weight), and the category was set to “standard female”. Maternal age in years and height to the nearest cm was entered. The mother stepped up on the scale and the Tanita® scale made a print of the measurement, which was added to the participant folder. Finally, information on exercise within the last 48 hours, time (mins) since last voiding (less than 30 mins), and time since last meal or drink was noted down as crude proxies of factors affecting hydration levels³⁴⁸.

Validation studies

The Tanita® measure of body composition has been assessed in healthy adults for measurement of TBW and FFM against the reference methods of DXA and underwater weighing³⁴⁹ as well as tritium and deuterium dilution methods^{349,350}. Impedance and measurements of TBW from dilution methods correlated well and both measures can give precise estimates of FFM, since FFM correlates strongly with TBW^{349,350}. Tanita® has also been assessed for the measurement of FM against the criterion method of the four-compartment model³⁵¹. Jebb and colleagues found strong positive correlations for both men and women within a wide distribution of BMI, but bias was negative for men (-0.9 kg) and positive for women (2.7 kg) and agreement was poor at the individual level³⁵¹. For the present study, this measure will only be used to describe the sample of mothers at the group level.

2.5.3 Skinfold thickness

Skinfold thickness was measured at two sites using a Holtain calliper (Crymych, UK), using the same procedure as for infants (section 2.4.4). For the mothers, the non-dominant side was used for measurements³⁴⁷. The subscapular skinfold measurement was obtained on a fold of skin angled approximately 45° from horizontal just under the inferior angle of the scapula. The triceps skinfold measurement was obtained on a vertical fold of skin at the midpoint of the upper arm, while the elbow was flexed to 90°. A measuring tape was applied from the acromion process to the olecranon process to find the midpoint, which was then marked with a pen. The reading was taken after approximately 2 seconds. The readings were done in triplicate to the last completed 0.2 mm and averaged to produce one measurement.

2.5.4 Waist circumference

The measurement of waist circumference can be used as an indicator of centrally deposited fat³⁴⁷. In the present study, the measurement was performed with the mother standing with arms at her sides, feet together and the abdomen relaxed. A measuring tape was applied horizontally in line with the umbilicus, and the mother was asked to exhale just before the reading was taken. The procedure of using umbilicus may deviate from some standard procedures, but was employed from the point of being easier to replicate on following visits in order to detect changes in waist circumference. The readings were done to the last completed 0.1 cm in triplicate and averaged to produce one measurement.

2.6 Breast-feeding practices and infant behaviour

As described in section 1.1.5, breast-feeding prevalence is strongly influenced by socio-demographic and behavioural factors. Infant behaviour can influence maternal breast-feeding practices as well as influence the demand for energy. For instance, an infant perceived to be fussy, might probe the mother to breast-feed more as a soothing technique, resulting in higher milk and energy intakes³⁵². On the other hand, crying more could increase energy expenditure, especially if the time spent on crying is replacing the time spent asleep³⁵³.

Essentially, exclusive breast-feeding is concerned with adequate milk intake to fulfil infant energy requirements, but how this milk is delivered in terms of frequency and duration of breast-feeds is determined by maternal breast-feeding practices. Furthermore, the maternal perception of her own breast-feeding practices influences her decision to persevere with or stop breast-feeding. There is a lot of research published on behavioural aspects of breast-feeding practices, and it is beyond the scope of the present thesis to explore this area fully. However, since behaviour and lactation physiology may be connected, it is relevant to include a few simple measures of infant behaviour and breast-feeding practices to test the Reilly-Wells hypothesis from a behavioural perspective (Chapter 7).

2.6.1 Baby Behaviour Diary

A diary, first developed by Barr and colleagues in 1988³⁵⁴, was adapted for the present study. The original diary was validated against audio-recordings, and significant moderately positive correlations were found between these and the diary records of crying time, although the study was quite small ($n = 10$ infants at 6 weeks of age)³⁵⁴. This diary has also been called “the Crying Diary” or “the Colic Diary”, and has been widely used for research in infant cry³⁵⁵⁻³⁵⁷ and colic behaviour^{358,359}. For the present study, the diary was named the “Baby Behaviour Diary” (BBD) (Appendix A.12). In the BBD, infant behaviour is divided into six categories; (1) Sleeping, (2) Awake and content, (3) Awake and active, (4) Fussy, (5) Crying and (6) Feeding, as defined in Table 2.3.







Table 2.3 Definitions of behavioural categories for the Baby Behaviour Diary.

Category	Description
Sleeping	Quiet with eyes closed.
Awake and content	Open eyes, orientation, minimal movement of arms and legs.
Awake and active	Actively moving of arms and legs e.g. to engage in play or respond to stimulus.
Fussy	Unsettled, irritable, restless or fractious and may be vocalising but not continuously crying
Crying	Periods of prolonged distressed vocalisation
Feeding	Latched on to breast or bottle of expressed breast milk, or in the process of eating complementary foods

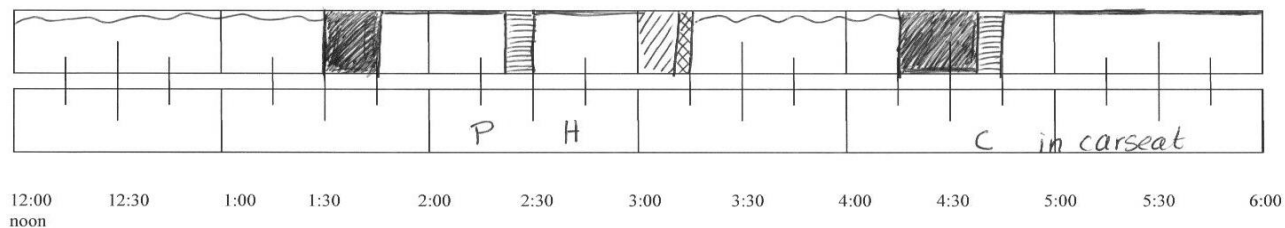
Each category has its own shading pattern, as shown in Figure 2.7. The time ruler has an upper and lower segment, for recording infant behaviour and care-giver activities with the infant, respectively. The time ruler corresponds to 24 hours, divided into four lines of 6 hours, which are then subdivided in hours and 15-minute segments (Appendix A.12).

The mother was told to record infant behaviour by using the appropriate shading pattern as accurately as she could within the segments of 15 minutes. However, it was acknowledged that due to the need to attend to the infant as well as other activities, the mother could not keep the diary on continuous prospective basis. Therefore, she was advised to fill it in, whenever she could find time to do so. This way of keeping the diary did introduce a risk of memory bias. However, this compromise was deemed necessary to ensure sufficient compliance and obtain the data.

The record is filled in by shading on the 'time rulers' using the appropriate type of shading. An example is given here. Note that activities or behaviour don't have to last for 15 minutes to be filled in. The length of shading in tells us how long they lasted for. If you can be accurate to within about 5 minutes, that will be accurate enough.

	Sleeping		Fussy: your baby is unsettled and irritable, and may be vocalising but not continuously crying
	Awake and content		Crying: periods of prolonged, distressed vocalisation
	Awake and active		Feeding

The top time ruler is for recording your baby's behaviour



The bottom time ruler is for recording your activities or those of other care-givers:

H = held or carried; C = bath or nappy change; P = play

Figure 2.7 Shading pattern and example of how to fill in the Baby Behaviour Diary.

The diary was kept for three consecutive 24-hour periods at each time-point between the second and third visits, on the days where there was no urine sampling (days 3, 4 and 5). From the BBD, data were obtained on the frequency and duration of breast-feeds as they had been prospectively recorded by the mother over the three days. These were averaged to produce an average frequency and duration at each time-point.

2.6.2 Breast-feeding practices

To devise and validate a measure of breast-feeding practices is a very labour intensive process and this was beyond the scope of the First-Feed study. Instead, a questionnaire was adapted from a large-scale American questionnaire.

The Infant Feeding Practices Study

The American Centers for Disease Control and the Food and Drug Administration have conducted the Infant Feeding Practices Study (IFPS) I (1993-1994) and II (2005-2007). The main objectives of IFPS-II were to evaluate the effect of a national breast-feeding promotion campaign that ran from 2004 - 2006 as well as evaluating the effects of the various changes in policies, information and education relating to infant feeding choices that had taken place in the intervening period³⁶⁰. The IFPS-II was a longitudinal postal questionnaire survey with approximately 4,900 pregnant women enrolled during their third trimester. They were sent 12 questionnaires from time of enrolment through to 12 months post-partum. The questionnaires explored a wide range of aspects of infant feeding practices, infant health and infant care³⁶¹. All the survey questions from this questionnaire study had been extensively reviewed.

The questionnaires were divided into various modules, and the modules related to breast-feeding practices were adapted for the First-Feed study. The adaptations made involved changes of spelling (for instance changing “mom” to “mum”) and omitting sections of the questionnaire concerned with brands of breast-pumps and breast-pump strategies, since these were deemed less important for the present study. Questions on initial breast-feeding (the first 2 weeks after birth) were included as part of the background questionnaire (see Figure 2.2). These were answered retrospectively on visit 1. All questionnaires used in the First-Feed study can be found in Appendix A.4, A.10 and A.14, but not all results from these are reported in the present thesis.

Breast-feeding practices questionnaire

The adapted questionnaire for current breast-feeding practices consisted of 26 items divided into four modules concerning breast-feeding at present, breast-feeding in future, breast-feeding attitudes and sleeping arrangements. The questionnaires were completed by the mother at the first visit of each time-point, at the mid-way visit, and at 9 months (sent by post) and follow-up visit, if the mother was still breast-feeding. It was emphasised that the questionnaires needed to be filled in before the three days of recording infant behaviour during the 1st and 2nd time-points, so that the responses in the questionnaires were not influenced by what the mother had just recorded in the BBD. In this way, the questionnaires were thought to reflect how the mother perceived the breast-feeding practices, while the BBD actually recorded the breast-feeding behaviour in terms of frequency and duration.

2.7 Statistical methods

2.7.1 Power and sample considerations

A reference study

To decide how many participants would be needed for the First-Feed study, it was necessary to consider the main hypothesis to be tested in the present study. The null hypothesis of the primary research question was no significant change in breast milk intake with duration of lactation, with the primary outcome variable³⁶² being daily milk intake. For this hypothesis to be tested, the number of participants needed would be based on the number of repeated measurements needed to detect a possible change in milk intake and from the 1st to the 2nd time-point using a paired t-test.

Previous studies which could be used to base a power calculation using isotopic methods to measure milk intake have either been cross-sectional^{28,363}, have not had an age span appropriate to guide the present study²⁷⁹, or they have not studied exclusively breast-fed infants³⁶⁴, as defined by WHO². Among the identified longitudinal studies measuring breast milk intake in exclusively breast-feeding infants, one did have a sufficient age span, but this study used the test-weighting method³⁶⁵.

Power calculation based on changes in milk volume intake

For the present study, it was decided to use the largest evidence base available in exclusively breast-fed infants to guide the power calculation, which was the systematic review by Reilly and colleagues³, described in section 1.2.4. In this review, the weighted mean \pm SD milk intakes based on cross-sectional studies were 779 \pm 40 g/d and 894 \pm 87 g/d at 3 - 4 and 6 months of age, respectively³, hence the difference between the cross-sectional time-points was 115 g/d, corresponding to an increase in energy requirements of 299 kJ/d (given a milk energy content of 2.6 kJ/d). This was considered a reasonable increase in milk intake matching an increase in estimated energy requirements of 294 kJ/d and 266 kJ/d for girls and boys, respectively, based on the FAO/WHO/UNU references for energy requirements⁷.

Since the data to base this power calculation were not paired, the power calculation would need to be performed as for two independent samples. The effect size (Cohen's d)³⁶⁶ was calculated to 1.322, and based on a power of 0.9 with a significance level of 0.05, this gave a sample size of 14 participants in each group, if the study had been cross-sectional³⁶⁷.

With a longitudinal study, the variation in the data would be smaller due to the measurements being paired, thus it would be more than sufficient to have paired successful measurements from 28 infants who would be exclusively breast-feeding at both time-points. Therefore, a sample size of 28 infants with successful repeated measurements was conservatively chosen as the population size to aim for in the present study. However, it was important also to account for infants not being exclusively breast-feeding at the 2nd time-point, for infants being excluded from the study, and for unsuccessful measurements. Therefore, a sample size of 60 recruited infants was deemed reasonable, expecting that about half of these would complete with successful measurements at two time-points.

2.7.2 General considerations on data-handling and statistics

Data entry and verification

The isotopic data were obtained and verified as described in section 2.3.5.

The anthropometric data and background information on socio-economic status, health and other participant characteristics were entered in one spreadsheet whilst the study was being conducted to be able to check any inconsistencies with the participants at the time. For instance, regarding initial breast-feeding problems (section 7.2.3), the option of “I had no problems” had to be ticked, if there were no other options ticked for this question. All of these data were then verified with the original paper records after the study had ended.

The breast-feeding questionnaire and the BBD were entered by Ms Eva Miriam Craig. These data entries were verified by a column-by-column check of each question response variable for extreme outliers, and a random sample of 10% of the entered questionnaires was verified with the paper records with no errors found. Finally, both the breast-feeding questionnaire and the BBD were checked for inconsistencies. For instance, from the BBD, the minutes spent in each category were added up to produce a check sum of 1440 min (24 hrs x 60 min). Once all data had been verified, they were transferred to PASW Statistics 18.0 (SPSS® inc., New York, US) for statistical analyses. Throughout the present thesis a significance level of $P < 0.05$ was used.

Descriptive statistics

In Chapters 3 - 7, results of the First-Feed study will be presented and discussed. For descriptive purposes, categorical data were described with frequency (%), continuous normally distributed data were summarised using arithmetic mean \pm SD³⁶⁸, and data which were not normally distributed were summarised using median (min - max). The exception was infant age/time post-partum which was not normally distributed due to the study design. Normality was explored using the Shapiro-Wilk test and histograms with assessment of skewness and kurtosis.

Inferential statistics

In general, the parametric tests used in the present thesis are fairly robust to minor deviations from normality³⁶², so when deciding whether to use parametric or non-parametric tests, it was also considered whether the variable to be analysed was a variable that could have been expected to be normally distributed had the sample size been larger. Changes over time were tested for significant changes using paired t-tests, and variables at individual time-points were tested against literature/reference values using one-sample t-tests.

Differences between sexes and feeding groups (section 3.2.4) in scale (continuous) variables were tested using independent t-test with Levene's test of homogeneity or Mann Whitney's U test if data were not normally distributed. Differences between categorical variables were tested using the χ^2 -test. Pearson (parametric) or Spearman (non-parametric) correlations were used to explore and quantify relationships between variables³⁶².

CHAPTER 3 PARTICIPANTS IN THE STUDY

3.1 Characteristics, infant anthropometry and growth

The present chapter describes the results of the First-Feed study in terms of participation, and socio-demographic characteristics, including maternal anthropometry and body composition, and it explores anthropometry and growth of the infants included in the study.

When interpreting the results of any research study, it is important to do so with an appreciation of the methodologies used. When performing anthropometric measurements in a field study, the measurements can be prone to imprecision and possibly also inaccuracy. Therefore, the present chapter also includes considerations on anthropometry measurement errors, and evaluates intra-observer variation of the anthropometric measurements that were most prone to this error.

3.1.1 Participation and socio-demographic characteristics

Strong associations between breast-feeding prevalence and socio-demographic factors were described in section 1.1.5. These associations vary with provenance, and, for the developed world, higher breast-feeding prevalence is associated with higher socio-demographic status backgrounds, including higher education levels, higher maternal age at primiparity, and more health-related behaviours. Breast-feeding studies often risk under-representing the poorer social groups, as mothers from these groups may not have resources to participate. This risk of unrepresentative sampling was high in the present study, particularly as mothers had to have persevered with exclusive breast-feeding for the first 15 weeks post-partum to be eligible for inclusion in the First-Feed study. Within the Scottish culture this practice is mostly limited to mothers from higher socio-demographic status backgrounds. Therefore, it was important to describe the socio-demographic characteristics of the participants in the present study.

3.1.2 The Reilly-Wells hypothesis on infant size and growth

The systematic review by Reilly and colleagues identified a shortfall in energy supply from milk intake during exclusive breast-feeding at 6 months of age³. One proposed explanation was that infants, who were exclusively breast-fed at 6 months of age, were unusually small⁴ and/or growing unusually slowly relative to the UK 1990 reference data²⁹⁶, which was the growth reference used in the UK at the time of the review. The studies included in the systematic review had not published growth data for these infants in a way that allowed a formal analysis of this matter³.

If the infants in the present study were unusually small in size and/or growing very slowly, then milk intake could be relatively low and still provide sufficient energy for these infants, but the results would not be applicable to the general population of infants. Therefore, the present study included infant anthropometry measurements to obtain a comprehensive set of data on infant size and growth, with the objective of comparing this data with a reference population of healthy infants that are growing “normally”.

3.1.3 The World Health Organization Child Growth Standards

Growth references

The growth reference chosen for the present study was the WHO Child Growth Standards³⁶⁹, derived from the WHO MGRS³⁷⁰. The idea for the MGRS was based on the acknowledgement that otherwise widely used reference data merely describes infant growth in a given setting^{371,372}, as was also the case for the UK 1990 reference²⁹⁶. This and other commonly used reference data, like the American National Center for Health Statistics (NCHS) growth reference from 1977³⁷³, and the Centers for Disease Control and Prevention (CDC) growth reference from 2000³⁷⁴, were generated from pooled anthropometry data on apparently healthy growing infants and children collected over several decades. Ignoring the effect of changes in infant growth over the decades, these references were also based on data from infants that were largely formula-fed or mixed-fed. The acknowledgement that growth in infancy is influenced by feeding mode¹³⁹⁻¹⁴⁸ created the need for a growth reference, where breast-feeding was the predominant feeding mode.

The World Health Organization Child Growth Standards

The MGRS was designed as a population study in 6 countries (Brazil, Ghana, India, Norway, Oman and USA), conducted between 1997 and 2003, where the inclusion criteria were concerned with optimal conditions for infant growth and development³⁷⁵. Among the inclusion criteria, infants had to be exclusively or predominantly breast-fed for at least 4 months³⁷⁶. By obtaining a comprehensive data set on anthropometry under these conditions, the WHO Child Growth Standards were intended to be prescriptive for normal growth of healthy infants under optimal conditions³⁷⁷. The MGRS consisted of a longitudinal component with anthropometry measurements of infants 0 - 24 months and a cross-sectional component of children aged 18 - 60 months. The WHO Child Growth Standards were published in April 2006³⁶⁹, and they were adopted as growth reference in the UK in January 2010³⁷⁸.

3.1.4 Precision of anthropometric measurements

When anthropometric measurements are performed they are affected by many sources of variation (errors) which reduce precision. These errors include biological sources of variation (e.g. day-to-day variation), as well as the technical error of each apparatus used for the measurements, which is illustrated in validation studies where repeated measurements are performed on calibration blocks or weights. For the present study, the same equipment was used throughout the study, but none of the equipment was re-calibrated during the study period.

Intra- and inter-observer variation

Observers also introduce variation into the measurements. In the MGRS, anthropometrists received extensive training, and their measurements were evaluated against peers at every reading as well as being evaluated against an expert for re-training purposes at regular intervals throughout the study period. This was to reduce the imprecision caused by intra- and inter-observer variation³³⁸. During the routine data-collection, the procedure was to have two anthropometrists present at each visit, each taking their readings and then comparing them to obtain consensus for each measurement. Hence, the MGRS included anthropometry data of very high quality³⁷⁹.

In the First-Feed study, all anthropometry measurements were performed by one researcher, the author, and therefore there was no inter-observer variation in the present study. However, intra-observer variation was a real concern, which included variations in the positioning of the participants as well as the way the researcher handled the equipment and performed the readings. This variation is particularly an issue with infant anthropometry measurements, because infants rarely cooperate fully when the measurements are performed due to the spontaneous nature of their body movements. The researcher had no formal training, although she had some previous experience in performing anthropometric measurements on infants. Therefore, it was a secondary objective in the present study to assess intra-observer variation in order to appreciate the precision of anthropometric data obtained.

3.1.5 Aims of the present chapter

The aims of the present chapter were to:

- Describe participation in the First-Feed study.
- Characterise the participants in terms of socio-demographic factors and maternal anthropometry.
- Describe infant anthropometry and test if infants were growing normally relative to the WHO Child Growth Standards.
- Evaluate intra-observer variation of infant anthropometric measurements.

3.2 Methods

3.2.1 Measures of socio-demographic status

In order to characterise a sample, it is useful to have area-based and household-based socio-demographic variables. As an area-based variable the Scottish Index of Multiple Deprivation (SIMD) was chosen. The SIMD is an index that includes 37 indicators of deprivation dividing Scotland into 6,505 individual data zones (small groups of street post codes) and ranks these after their degree of deprivation³⁸⁰. To determine the SIMD decile for each participant, the postcode was entered into the Scottish Neighbourhood Statistics website³⁸¹, and the SIMD decile as well as the individual rank were recorded as background information.

As a household-based measure of socio-demographic status, it seemed best to include the maternal factors, such as educational attainment, age and parity, which are known to be associated with duration of breast-feeding. This information was collected from the background questionnaire performed at visit 1. Maternal anthropometry was also measured at visits 1, 5 and 8 for descriptive purposes, as described in section 2.5.

3.2.2 Infant anthropometry

Infant anthropometry measurements were performed as described in section 2.4. Subsequently, weight, length and skinfold thicknesses were expressed as sex- and age-specific z-scores relative to the WHO Child Growth Standards³⁸². In principle, z-scores can be calculated as:

$$\text{z-score} = \frac{\text{Variable}_{\text{ind}} - \text{Variable Mean}_{\text{ref}}}{\text{Variable SD}_{\text{ref}}}$$

where the *Variable* is either weight, length, triceps or biceps skinfolds, and $\text{Variable}_{\text{ind}}$ is the individual value, while the mean_{ref} and SD_{ref} are reference values³⁴⁷. However, this method is only appropriate, if the data is normally distributed, and the WHO Child Growth Standards only showed normal distribution for length³⁸².

Instead, an alternative approach was taken, using a programme, WHO Anthro© Version 3 (WHO 2009), which can be downloaded free from the WHO website³⁸³. Data (name, date of birth, date of visits and length, weight, and skinfolds) from the First-Feed study were entered manually into this programme, which then calculated the sex- and age-specific z-scores.

A common cut-off for identifying infants at risk of malnutrition is beyond ± 2.0 z-scores³⁸⁴, where risk of underweight is defined as ≤ -2.0 z-scores in weight-for-age, risk of stunting is defined as ≤ -2.0 z-scores in length-for-age, risk of wasting is defined as ≤ -2.0 z-scores in weight-for-length, and risk of being overweight is defined as > 2.0 z-scores in weight-for-age. These cut-offs are also used when evaluating the performance of growth charts relative to each other in terms of identifying infants at risk of malnutrition³⁸⁵. The infants in the present sample were evaluated for risk of malnutrition using these cut-offs.

3.2.3 Evaluation of intra-observer variation

The anthropometric measurements, which were deemed prone to intra-observer variation, were performed on visit 1 and then repeated on a subset of 12 mothers and infants on visit 2, the following day. For the purpose of evaluating intra-observer variation, only data on the infants are reported below. All 12 mother-infant pairs were recruited for these extra measurements at the beginning of the study period, thus any effect of training as a result of conducting the study could not be assessed. The intra-observer variation was only evaluated from data obtained at the 1st time-point, so the effect of infant age on the intra-observer variation could not be assessed either. Given the number of measurements undertaken at each visit, one day was considered enough for the measurements to be blinded from the day before, but not enough to include any real changes caused by growth.

In the MGRS, standardisation sessions were carried out where anthropometrists measurements were standardised against an expert³⁸⁶, which were subsequently used to evaluate “the intra-observer technical error of measurement”³⁸⁶. Assuming that the technical error is negligible compared to observer variation, the intra-observer variation was calculated for the present study as:

$$TEM = \sqrt{\sum_{i=1}^N \frac{(M_{i1} - M_{i2})^2}{2 \times N}}$$

where M_{i1} and M_{i2} are the measurements done at visit 1 and visit 2, respectively, and N is the number of participants. From this equation, the difference between duplicate measurements will be within “ \pm this value” of TEM for two-thirds of the time³⁸⁶. The reported mean intra-observer variation for each anthropometric measurement for the six study sites in the MGRS was used for comparison.

3.2.4 Statistical methods

The participants in the First-Feed study were characterised using summary statistics as described in section 2.7.2. Participant drop-out and completion was described along with compliance to the visit protocol of the First-Feed study (section 2.2.3). Finally, infant feeding status at the 2nd time-point was categorised in feeding groups as either exclusively breast-fed, as defined by WHO², or complementary breast-fed.

Infant anthropometry was summarised as z-scores relative to the WHO Child Growth Standards³⁸². Inferential statistics were performed as described in section 2.7.2, including data-driven tests for differences between sexes (with the expectation of boys being bigger than girls) and feeding groups in variables of socio-demographic status and anthropometry (independent t- tests), and testing for changes over time in variables of anthropometry (paired t-tests). To test if the infants in the sample were unusually small, z-scores were tested for their difference from zero using one-sample t-tests, and paired t-tests of z-scores were used to test for longitudinal changes in z-scores, which could indicate a growth pattern different from the WHO Child Growth Standards. A one-sample t-test was used to test if the intra-observer variation in the present study was significantly different from the distribution of intra-observer variations obtained from the six centres in the MGRS ³⁸⁶.

3.3 Results

3.3.1 Participation

Figure 3.1 presents an overview of recruitment, participation and withdrawals of the First-Feed study. To recruit 60 mother-infant pairs, 146 mothers were approached, where 31 were not eligible, 34 did not respond and 21 declined to participate. Of the 60 recruited mothers, 10 were not included in the study; two could not be available for measurements at the 1st and/or the 2nd time-point, three had changed their mind about exclusive breast-feeding to 6 months, as they were going back to work before then, one mother changed her mind because she found the study too much work, one mother had concerns about her infant's health and three had started formula feeding in addition to breast-feeding, and did therefore not fulfil the inclusion criteria. No data were obtained from these 10 mother-infant pairs as they had not yet signed informed consent.

Of 50 mothers included in the study, one withdrew for personal reasons, and two were excluded due to maternal illness affecting the breast-feeding. Of these, two were excluded during the 1st time-point (visit 2), without any data on milk intake or energy balance being obtained. The third mother was excluded after a successful measurement at the 1st time-point, which was included in the data.

Furthermore, one mother was included for measurements at the 2nd time-point only. The completion rate was 47 out of 50 (94%) mother-infant pairs included, and 47 out of 60 (78%) mother-infant pairs recruited to the study.

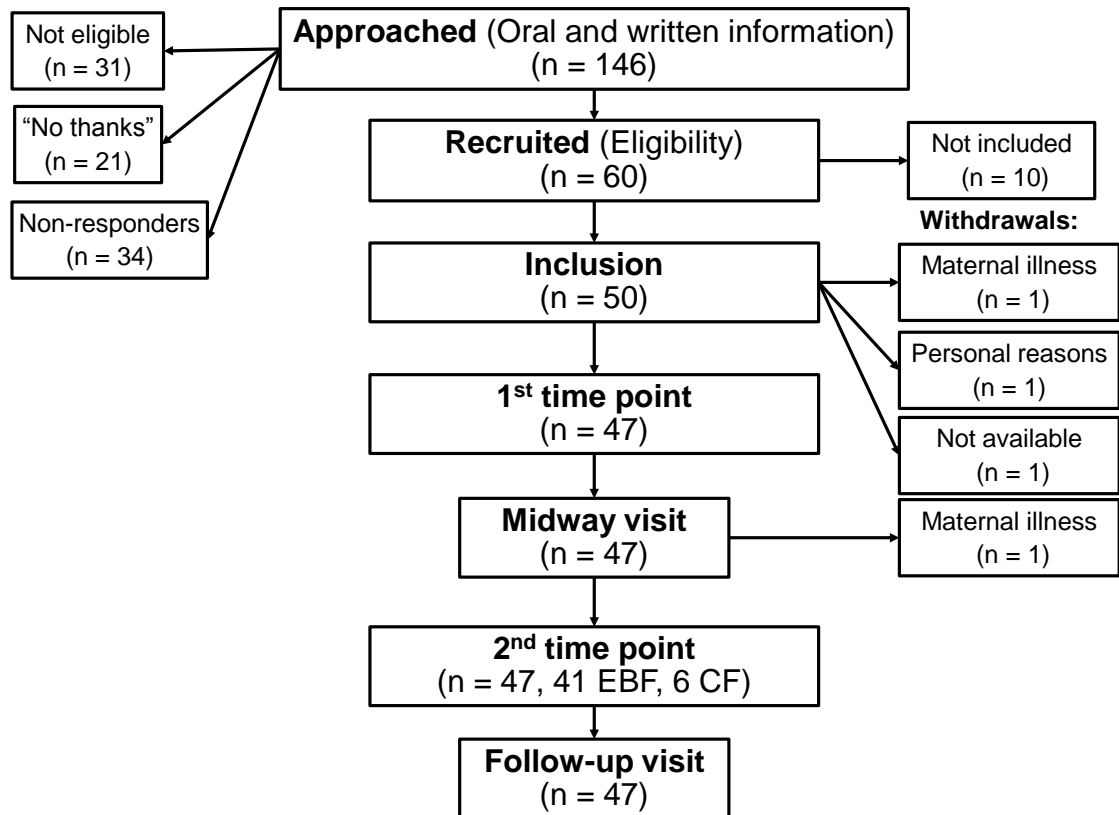


Figure 3.1 Flow-chart of recruitment, participation and follow-up in the First-Feed study.

EBF: Exclusive breast-feeding

CF: Partial breast-feeding

Observational feeding groups and differences between feeding groups

Of the 47 completed mother-infant pairs, 41 were still exclusively breast-feeding at the 2nd time-point, whilst six mothers had introduced complementary foods. Of those six, two mothers had introduced complementary foods around 20 - 21 weeks of age, and four had introduced complementary foods just prior to the 2nd time-point. However, during the week of measurements only three of those four had any intake of complementary foods.

These six mother-infant pairs were not significantly different from the exclusively breast-fed mother-infant pairs in terms of SIMD decile, maternal age, height, BMI or infant birth weight, infant weight at the 1st or the 2nd time-point (all $P > 0.05$).

The six mothers were supplied with a digital scale with an increment of 1g to measure all intakes of complementary foods during the week of measurement at the 2nd time-point. Median (min - max) daily intake of complementary foods ranged from 4 (0 - 8) g/d to 302 (182 - 344) g/d for five of the six infants. None of the infants received infant formula.

Since the group of infants that were complementary breast-fed was small, and the amount consumed of complementary foods was almost negligible, the First-Feed study is not sufficiently powered to fulfil the objective of exploring differences between feeding groups in terms of milk intake, energy balance and growth. Therefore, no further analysis of this is reported in the present thesis. However, for the publications arising from the present study and because it was important to adhere strictly to the feeding practice of exclusive breast-feeding, as defined by WHO², some tables will summarise data at the 2nd time-point both for exclusively breast-fed infants only and for all infants.

Infant age at each time-point

In total 370 home visits were performed; for a few of the participants it was more convenient to meet at the Queen Mother's Hospital for measurements. The visits had to be carefully planned relative to infant age. However, for the two time-points involving the DLW method, the three visits also had to be co-ordinated tightly so as not to compromise the DLW measurement. When this presented logistical challenges, some deviations from the visit protocol had to be accepted regarding infant age. If the 1st time-point had been conducted late, or the 2nd time-point needed to be conducted early for planning reasons (e.g. participants on holiday at 6 months of age), the mid-way visit was omitted. Therefore, only 40 participants had a mid-way visit. Figure 3.2 presents the distribution of infant age at the beginning of the two time-points.

The ages were 13 - 21 weeks and 20 - 27 weeks for the 1st and 2nd time-points, respectively. This variation in infant age introduced a factor of variation in the data in addition to the biological variation caused by variations in activity levels, body size and growth velocity etc. at any given age.

The median (min - max) interval between the 1st and 2nd time-points was 9.2 (4 - 12) weeks (n = 46). Eight infants had an interval shorter than 8 weeks. Two infants had a late 1st time-point due to being recruited late into the study.

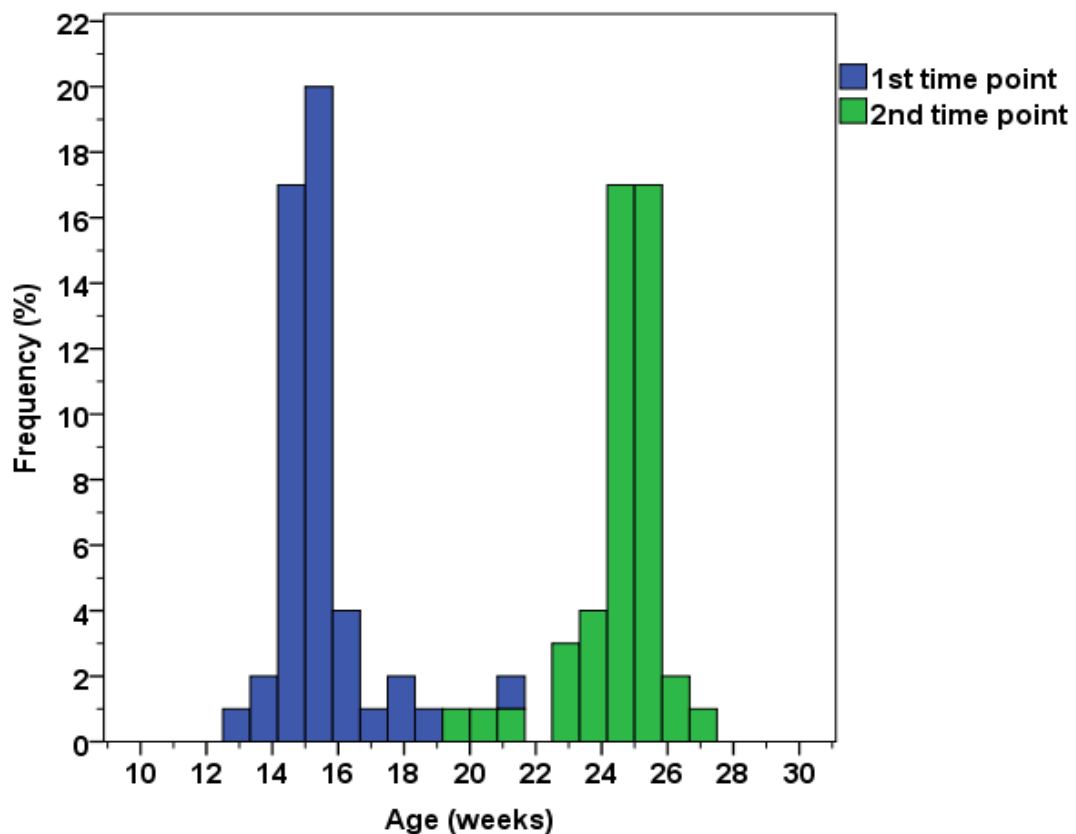


Figure 3.2 Distribution of infant age at the start of the 1st (n = 49) and the 2nd (n = 47) time-point.

Six infants had a 2nd time-point before 24 weeks of age either due to holidays, or because the mother wanted to start introduction to complementary foods at 24 - 25 weeks of age for convenience rather than 26 weeks of age (e.g. the mother going back to work at 26 weeks post-partum). Four infants had an interval longer than 10 weeks, where one infant had an early 1st time-point and two infants had a late 2nd time-point due to holidays, and one infant had the 2nd time-point repeated due to an unsuccessful dose administration.

3.3.2 Socio-demographic characteristics of the participants

Area-based measure of socio-demographic status

The distribution of SIMD deciles for the participants in the First-Feed study is shown in Figure 3.3. The median (min - max) SIMD decile was 8 (2 - 10). Thus, the majority of participants (36; 72%) lived in areas that were above the Scottish median in terms of degree of deprivation. Geographically, the mothers mainly resided within the Greater Glasgow area, but residences extended as far as Balfron, north of Glasgow, and Hamilton, south of Glasgow, and to Edinburgh in the east of Scotland and Greenock in the west of Scotland.

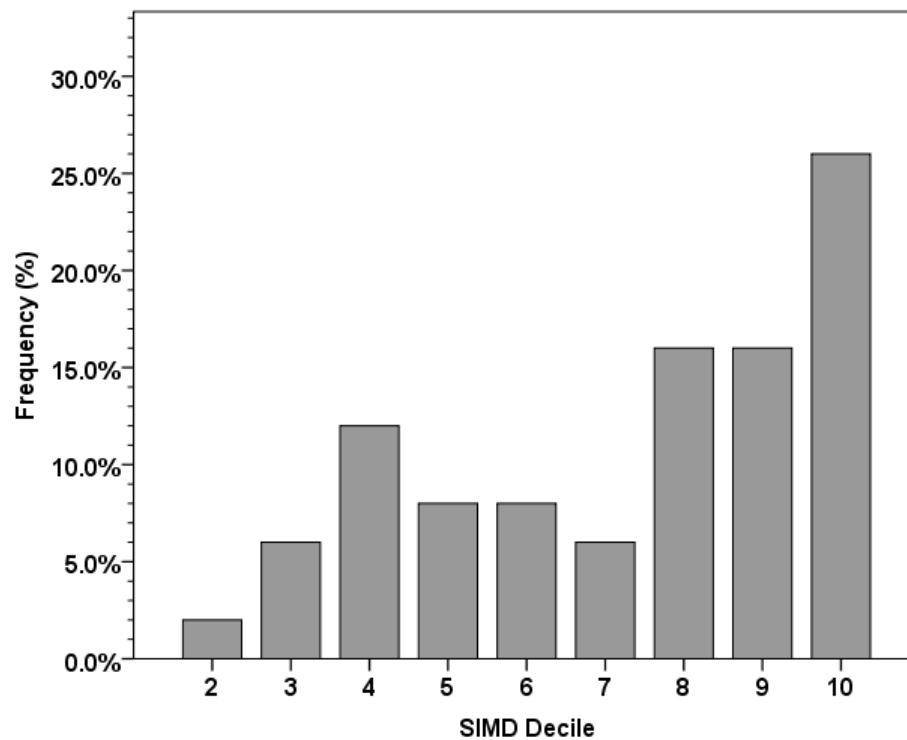


Figure 3.3 Distribution of SIMD deciles for included participants (n=50).

Household-based measures of socio-demographic status

Characteristics of the mothers in the present study are presented in Table 3.1. All mothers were non-smoking and were either married or living with a partner. The mean \pm SD maternal age was 33.7 ± 4.3 years ($n = 50$) at the time of inclusion in the study. They were well-educated with a high age compared to their parity. The majority were white European, but 5 (10%) were of mixed ethnicity or Asian.

Table 3.1 Maternal socio-demographic characteristics (frequency and %) of included and completed participants.

	Included (n = 50)		Completed (n = 47)	
	Frequency	%	Frequency	%
Educational attainment				
Higher grades ^a	1	2.0	1	2.1
College	4	8.0	4	8.5
University	45	90.0	42	89.4
Parity				
1 st child	35	70.0	32	68.1
2 nd child	10	20.0	10	21.3
3 rd child	4	8.0	4	8.5
4 th child	1	2.0	1	2.1

^a Equivalent to 12 years of school education

3.3.3 Maternal anthropometry

Maternal anthropometry was measured at the 1st and 2nd time-points and at one year post-partum (Table 3.2). Maternal mean BMI was close to the cut-off between healthy weight and overweight, according to the international BMI classifications³⁸⁷.

Table 3.2 Characteristics of maternal anthropometry (mean \pm SD).

	1 st time point (n = 49)	2 nd time point (n = 47)	Follow-up (n = 47)
Time post-partum, weeks	15.4 \pm 1.3	24.5 \pm 1.3	52.0 \pm 0.5
Height, cm	165.0 \pm 7.5 ^a	-	-
Weight, kg	68.3 \pm 10.4	67.4 \pm 10.2	66.0 \pm 10.2 ^d
BMI, kg/m²	25.1 \pm 3.9	24.9 \pm 4.0	24.3 \pm 4.1 ^d
Body Composition			
FM, kg	23.3 \pm 7.5	22.8 \pm 7.4 ^d	21.3 \pm 7.4 ^d
FFM, kg	45.0 \pm 3.6	44.6 \pm 3.3 ^b	44.7 \pm 3.4 ^b
Fat %	33.3 \pm 6.0	33.0 \pm 6.1 ^c	31.4 \pm 6.3 ^d
Skinfold			
Subscapular, mm	21.2 \pm 8.3	20.1 \pm 8.2 ^c	18.1 \pm 7.4 ^d
Triceps, mm	25.4 \pm 6.9	24.7 \pm 7.8	21.7 \pm 6.7 ^d
Skinfold sum, mm	46.6 \pm 13.8	44.8 \pm 14.5 ^b	39.8 \pm 13.1 ^d
Waist circumference, cm	91.3 \pm 8.9	90.0 \pm 8.4 ^d	88.1 \pm 9.3 ^d

All data, except time post-partum, were normally distributed.

^a n = 50.

Difference from 1st time-point (paired t-test), n = 46; ^b P <0.05, ^c P <0.001, ^d P <0.0001

BMI: Body Mass Index, FM: Fat mass, FFM: Fat free mass

At the 1st time-point, 22 mothers (45%) were overweight or obese (BMI \geq 25). At one year post-partum, 19 mothers (40%) were still overweight or obese. Overall, there were small, but significant improvements in all maternal anthropometric variables from the 1st time-point to one year follow-up (all P <0.05). Given that there is great variability in post-partum changes in maternal anthropometry³⁸⁸, the present study was not expected to be powered to explore relationships between indicators of maternal nutritional status and lactation performance.

3.3.4 Infant characteristics at birth

Information on birth size was obtained from the “red book” (The UK Personal Child Health Record); a booklet given to the mother, in which primary care professionals record measurements of the individual infant.

All mothers had information on birth weight measured either imperially (in pounds and ounces) or metrically (in kilograms); 22 mothers had information on birth length and nine mothers had information on head circumference (Table 3.3). Apart from the boys having higher birth weight than the girls, there were no significant differences between sexes.

Table 3.3 Birth characteristics (mean \pm SD) of included infants.

	Boys		Girls	
	n	mean \pm SD	n	mean \pm SD
Gestational age at birth, weeks	25	40.3 \pm 1.0	25	40.0 \pm 1.1
Birth weight, kg^a	25	3.8 \pm 0.4	25	3.5 \pm 0.4 ^b
Birth length, cm	12	52.9 \pm 3.0	10	51.6 \pm 3.1
Head circumference, cm	6	35.6 \pm 1.7	3	34.3 \pm 0.8

Normality was ascertained for all variables, except head circumference for girls.

^a Imperially measured weight was converted using 1 pound = 453.59g and 1 ounce = 28.35g.

^b Independent t-test for difference between sexes, $P = 0.021$.

3.3.5 Infant anthropometry during the study

Missing data-points

As described in Chapter 2, some of the infant anthropometry measurements were missed or omitted for various reasons, and Table 3.4 gives a condensed account of the reasons for the missing data-points. The low numbers of weight measurements performed at visits 2 and 6 were due to a protocol change mid-way through the study period, where this measurement was included at these visits (section 2.2.3).

Table 3.4 Numbers of missed or omitted infant anthropometry measurements at visits 1 to 8.

	1	2	3	4	5	6	7	8
Weight	49 ^a	24 ^b	43 ^{a,c,d}	41 ^{a,c,e,f}	47 ^{c,g}	24 ^b	44 ^{c,g,h}	47 ^{c,g}
Length	48 ^{a,j}	12 ^k	43 ^{a,c,d}	41 ^{a,c,e,f}	47 ^{c,g}	ND	44 ^{c,g,h}	47 ^{c,g}
Knee-heel-length	48 ^{a,j}	ND	42 ^{a,c,d,j}	40 ^{a,c,e,f,j}	47 ^{c,g}	ND	42 ^{c,g,h,jx2}	47 ^{c,g}
Skinfolds	49 ^a	12 ^k	43 ^{a,c,d}	41 ^{a,c,e,f}	47 ^{c,g}	ND	44 ^{c,g,h}	47 ^{c,g,i}

^a 1 included late into the study

^b n = 24 done due to change of practice

^c 2 excluded from the study

^d 4 unsuccessful dose administrations

^e 1 not done due to illness

^f 5 not done due to age; 1st time-point was late or 2nd time-point was early

^g 1 more excluded from the study

^h 3 unsuccessful dose administrations

ⁱ Triceps skinfolds n = 46, as one measurement was abandoned due to infant fatigue

^j 1 measurement not done due to malfunctioning equipment

^k n = 12 was subset for evaluation of measurement variation

Other reasons for missing data-points were due to the variability of infant age at the 1st and 2nd time-points which led to the mid-way visit being omitted, and unsuccessful dose administrations which cancelled the last visit of the week of measurement (visit 3 and 7 at the 1st and 2nd time-points, respectively).

Initially, the infant weight measurement from the day before was used to determine initial enrichment obtained from the dose administration. However, when it was realised, that day-to-day variation in infant weight might be great enough to affect this calculation, the practice was changed, and the infant was reweighed on visit 2. After conducting the study, the difference between weights of day -1 and day 0 was calculated for those who had their weight measured on both days to be 20 g (equivalent to the increment of the scale) at the 1st time-point (n = 24) and 0 g at the 2nd time-point (n = 24).

Infant growth for the whole sample

The summary statistics of infant anthropometry are displayed for boys and girls and all infants in Table 3.5. Overall, the infants increased significantly in weight, length and knee-heel-length from one visit (or time-point) to the next (all $P < 0.0001$), as expected. For instance, on average the infants increased their weight by 39% from the 1st time-point to the follow-up at 1 year of age.

During the week of measurement the infants' weight gain was 187 ± 80 g ($P < 0.0001$) and 156 ± 358 g ($P = 0.0059$) at the 1st and 2nd time-points, respectively. The crown-heel-length did not change significantly during the weeks of measurements, but knee-heel-length increased significantly at the 1st time-point with a mean \pm SD paired difference of 2.7 ± 4.7 mm ($P = 0.0006$, $n = 42$). The changes in skinfold measurements during the study were not significant.

Differences between sexes

The boys were significantly heavier than girls at the 1st and 2nd time-points, and they had a slightly longer knee-heel-length at the 2nd time-point. By coincidence, the boys were a few days older at the follow-up visit. All other differences between sexes were not significant, but show a tendency of boys being heavier, longer and with higher triceps skinfolds, while girls tended to have higher subscapular skinfolds.

There was no difference in growth between boys and girls, even when adjusted for infant age and time interval between measurements (data not shown).

Table 3.5 Infant age and anthropometry (mean \pm SD) during the First-Feed study.

	1 st time point			Mid-way			2 nd time point			Follow-up		
	Girls (n = 25)	Boys (n = 24)	All (n = 49)	Girls (n = 21)	Boys (n = 20)	All (n = 41)	Girls (n = 24)	Boys (n = 23)	All (n = 47)	Girls (n = 24)	Boys (n = 23)	All (n = 47)
Infant age, Weeks	15.6 \pm 1.5	15.1 \pm 0.9	15.4 \pm 1.3	20.1 \pm 0.5	20.1 \pm 0.6	20.1 \pm 0.5	24.5 \pm 1.4	24.6 \pm 1.3	24.5 \pm 1.3	51.9 \pm 0.5	52.2 \pm 0.5 ^c	52.0 \pm 0.5
Weight, kg	6.30 \pm 0.64	6.72 \pm 0.78 ^c	6.51 \pm 0.74	6.83 \pm 0.73	7.27 \pm 0.88	7.05 \pm 0.83 ^d	7.37 \pm 0.75	7.84 \pm 0.91 ^c	7.60 \pm 0.86 ^d	9.63 \pm 0.97	9.97 \pm 1.15	9.80 \pm 1.06 ^d
Length, cm	61.5 ^a \pm 2.2	62.3 \pm 2.1	61.9 ^b \pm 2.2	63.1 \pm 1.7	64.1 \pm 2.0	63.6 \pm 1.9 ^d	65.1 \pm 2.0	66.1 \pm 2.0	65.6 \pm 2.0 ^d	74.0 \pm 2.8	74.4 \pm 2.9	74.2 \pm 2.8 ^d
Knee-heel-length, cm	15.9 \pm 0.8	16.3 \pm 0.9	16.1 \pm 0.9	16.6 \pm 0.8	16.9 \pm 0.6	16.7 \pm 0.7 ^d	16.9 \pm 0.8	17.6 \pm 0.8 ^c	17.2 \pm 0.8 ^d	20.1 \pm 1.0	20.1 \pm 1.0	20.1 \pm 0.9 ^d
Skinfolds												
Subscapular, mm	6.5 \pm 1.6	6.2 \pm 1.3	6.4 \pm 1.4	6.3 \pm 1.0	6.0 \pm 1.6	6.1 \pm 1.3	6.6 \pm 1.2	6.1 \pm 1.5	6.3 \pm 1.4	6.8 \pm 1.7	6.5 \pm 1.6	6.7 \pm 1.6
Triceps, mm	8.4 \pm 1.5	8.9 \pm 1.4	8.7 \pm 1.5	8.7 \pm 1.9	8.9 \pm 2.1	8.8 \pm 2.0	9.3 \pm 2.1	9.3 \pm 1.7	9.3 \pm 1.9	8.7 \pm 1.9	9.3 \pm 1.8	9.0 \pm 1.9

All data were normally distributed, except subscapular skinfold at the mid-way and follow-up visits, where there was one outlier (not the same infant). If outliers were removed, they were normally distributed, but the outliers were included in all analysis.

^a n = 24

^b n = 48

Difference between sexes (independent t-test); ^c P <0.05.

Difference from previous measurement (paired t-test); ^d P <0.001.

3.3.6 Growth relative to World Health Organization Child Growth Standards

The summary z-scores for boys, girls and the whole sample are presented in Table 3.6. As per definition, the WHO Child Growth Standards are normalised distributions with mean z-scores of 0.00 for each sex. Therefore, there were no significant differences between boys and girls in the present sample for any of the z-scores (confirmed by independent t-tests). The girls had a significantly positive weight-for-length z-score at the 2nd time-point and at 1 year, where it was also the case for the boys. The girls also had a significantly positive weight-for-age z-score at 1 year. By contrast the boys had a significantly negative length-for-age z-score at the mid-way visit (20 weeks) and again at 1 year. BMI-for-age was significantly positive at 1 year for both boys and girls. Triceps-for-age was significantly negative at first, but became positive by 1 year. Subscapular-for-age were negative throughout, but only significantly so in the first 6 months.

Prevalence of malnutrition risk

Using cut-offs³⁸⁴ as defined in section 3.2.2, no infants were identified as at risk of being underweight (≤ -2.0 z-scores in weight-for-age) at the 1st or 2nd time-points, whilst one infant was identified as at risk of being underweight at the 1 year follow-up. One infant was identified as at risk of stunting (≤ -2.0 z-scores in length-for-age) at the 2nd time-point, and a further three infants were at risk of stunting at 1 year. One infant was at risk of wasting (≤ -2.0 z-scores in weight-for-length) at the 1st time-point, but there were none at the following visits. Finally, defining risk of being overweight as >2.0 z-scores in weight-for-length, the study identified one infant at the 1st time-point, two infants at the 2nd time-point and three infants at 1 year.

Table 3.6 below summarises the z-scores and the distributions of the z-scores are displayed for boys and girls together with the WHO Child Growth Standard for the 1st time-point (Figure 3.4), the 2nd time-point (Figure 3.5) and the follow-up visit (Figure 3.6), respectively.

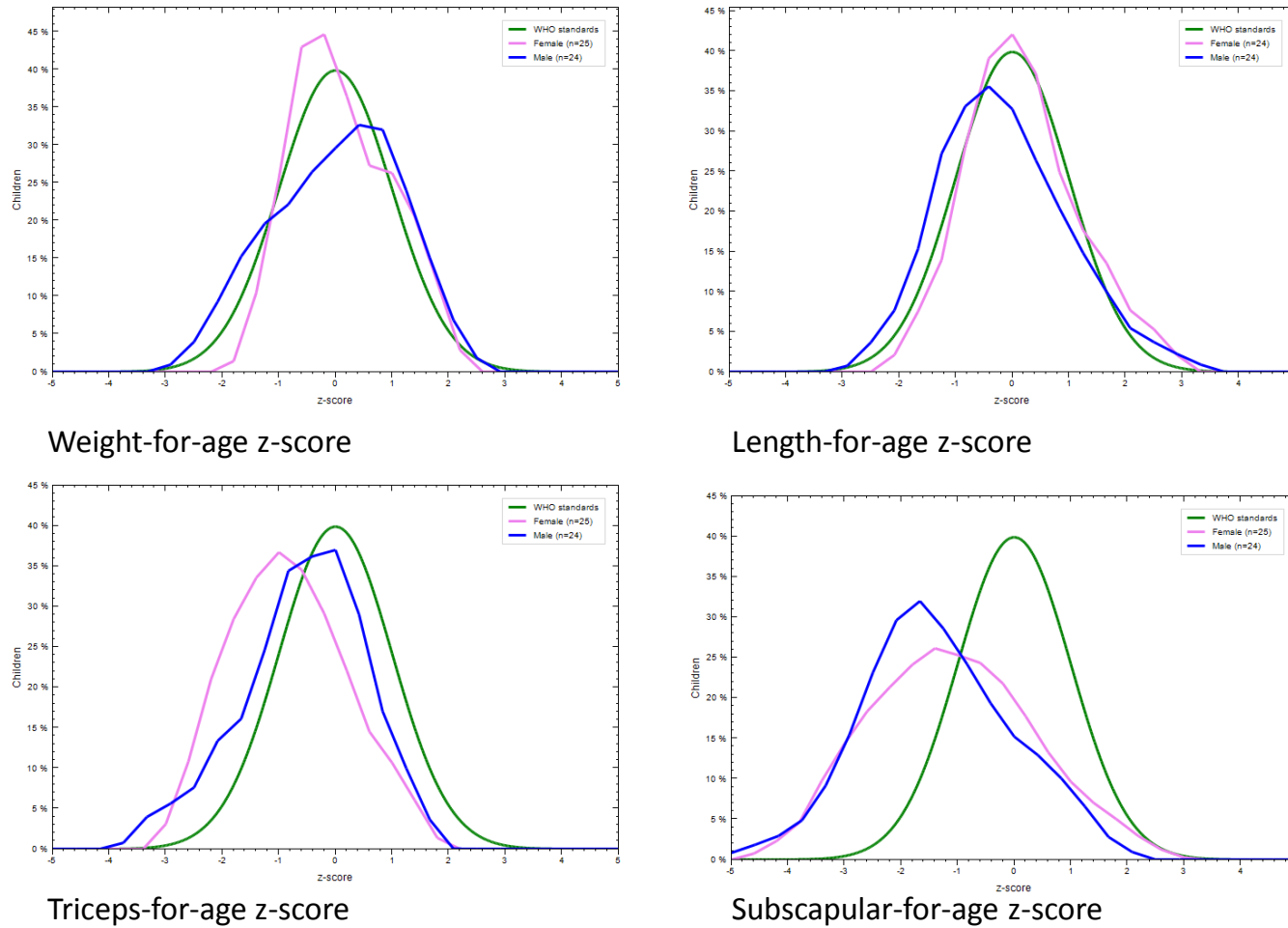
Table 3.6 Sex- and age-specific z-scores (mean \pm SD) relative to the WHO Child Growth Standards.

	1 st time point			Mid-way			2 nd time point			Follow-up		
	Girls (n = 25)	Boys (n = 24)	All (n = 49)	Girls (n = 21)	Boys (n = 20)	All (n = 41)	Girls (n = 24)	Boys (n = 23)	All (n = 47)	Girls (n = 24)	Boys (n = 23)	All (n = 47)
Weight-for-length, z-score	0.04 ± 0.93	0.19 ± 1.10	0.11 ± 1.01	0.27 ± 0.91	0.31 ± 1.09	0.29 ± 0.99	0.36 $\pm 0.84^a$	0.37 ± 0.95	0.36 $\pm 0.89^b$	0.76 $\pm 0.87^c$	0.68 $\pm 0.98^b$	0.72 $\pm 0.92^c$
Weight-for-age, z-score	0.11 ± 0.81	0.01 ± 1.05	0.06 ± 0.93	0.07 ± 0.84	-0.13 ± 1.10	-0.03 ± 0.97	0.21 ± 0.87	-0.03 ± 1.04	0.09 ± 0.96	0.57 $\pm 0.79^b$	0.25 ± 1.06	0.41 $\pm 0.93^b$
Length-for-age, z-score	0.21 ± 0.91	-0.16 ± 1.05	0.03 ± 0.99	-0.12 ± 0.76	-0.52 $\pm 0.97^a$	-0.31 $\pm 0.88^a$	0.02 ± 0.95	-0.41 ± 1.02	-0.19 ± 1.00	0.04 ± 1.11	-0.54 $\pm 1.19^a$	-0.24 ± 1.17
BMI-for-age, z-score	0.00 ± 0.90	0.13 ± 1.10	0.06 ± 0.99	0.18 ± 0.91	0.22 ± 1.10	0.20 ± 0.99	0.27 ± 0.84	0.28 ± 0.96	0.27 $\pm 0.89^a$	0.75 $\pm 0.92^c$	0.77 $\pm 0.96^b$	0.76 $\pm 0.93^c$
Skinfolds												
Triceps-for-age, z-score	-0.74 $\pm 0.96^b$	-0.57 $\pm 1.00^a$	-0.66 $\pm 0.98^c$	-0.57 $\pm 1.11^a$	-0.50 ± 1.33	-0.53 $\pm 1.21^b$	-0.06 ± 1.12	-0.08 ± 0.97	-0.07 ± 1.04	0.27 ± 1.05	0.63 $\pm 0.96^b$	0.45 $\pm 1.01^b$
Subscapular-for-age, z-score	-1.19 $\pm 1.20^c$	-1.36 $\pm 1.22^c$	-1.27 $\pm 1.20^c$	-1.01 $\pm 0.93^c$	-1.53 $\pm 1.45^c$	-1.26 $\pm 1.22^c$	-0.64 $\pm 1.05^b$	-1.23 $\pm 1.36^c$	-0.93 $\pm 1.23^c$	0.01 ± 1.23	-0.14 ± 1.20	-0.06 ± 1.21

Data were normally distributed.

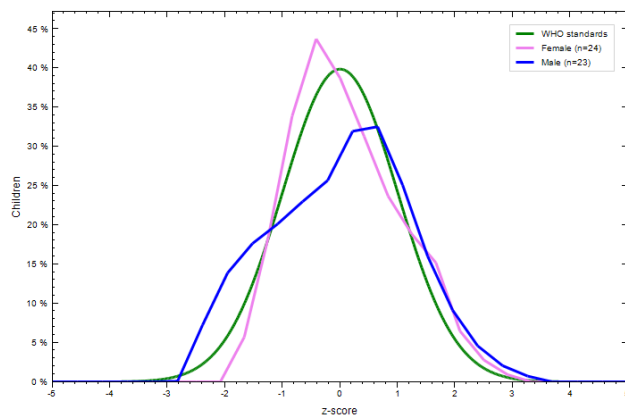
Significantly different from WHO Child Growth Standards (one-sample t-tests); ^a P <0.05, ^b P <0.01, ^c P <0.001

Figure 3.4 Distributions of age-specific weight, length and skinfold z-scores for each sex at the 1st time-point.

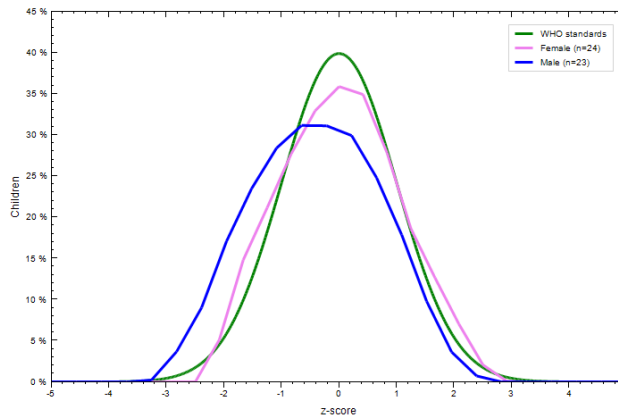


Green lines are WHO Standards, pink lines are girls and blue lines are boys. Graphs were generated from the WHO Anthro³⁸³ programme.

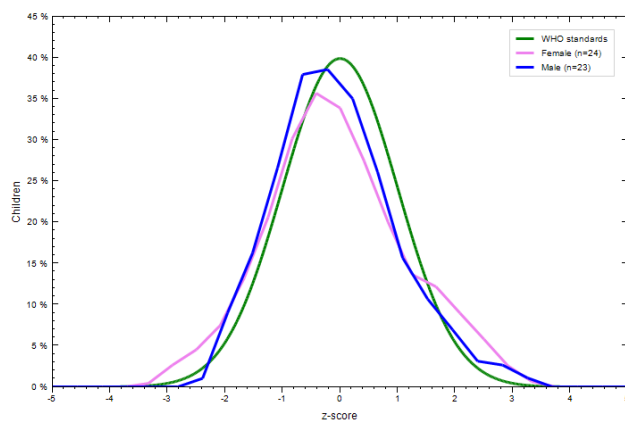
Figure 3.5 Distributions of age-specific weight, length and skinfold z-scores for each sex at the 2nd time-point.



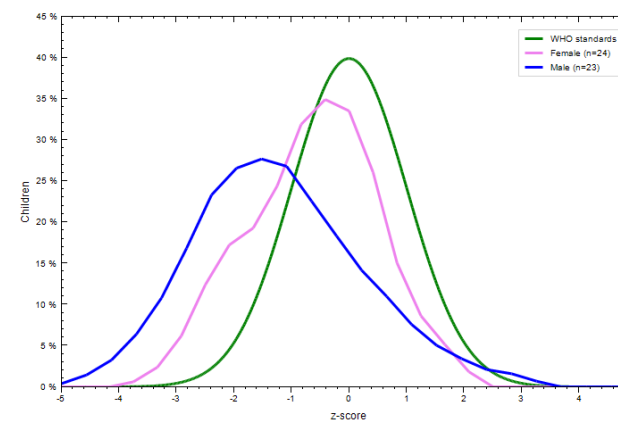
Weight-for-age z-score



Length-for-age z-score



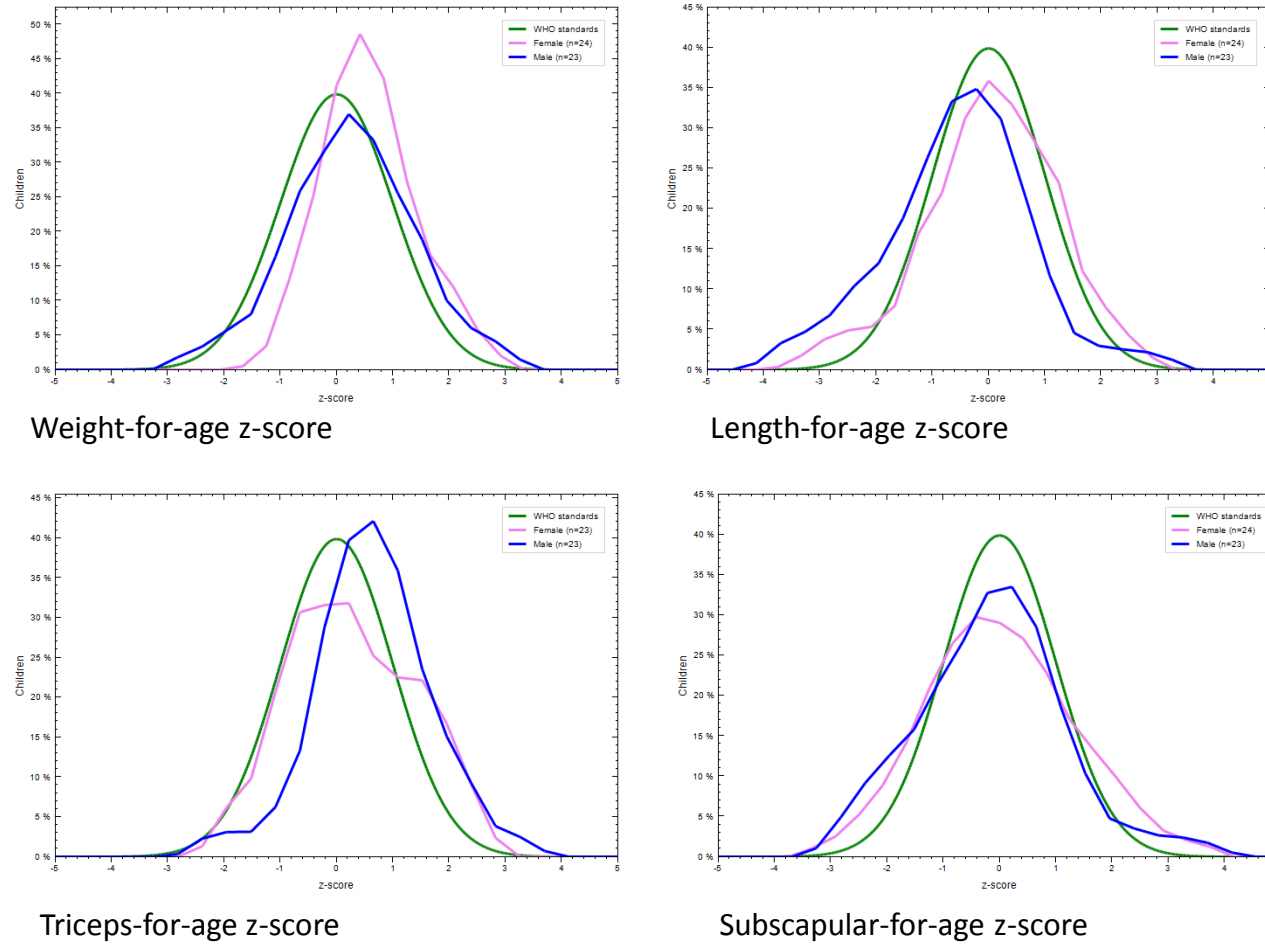
Triceps-for-age z-score



Subscapular-for-age z-score

Green lines are WHO Standards, pink lines are girls and blue lines are boys. Graphs were generated from the WHO Anthro³⁸³ programme.

Figure 3.6 Distributions of age-specific weight, length and skinfold z-scores for each sex at the 1 year follow-up.



Green lines are WHO Standards, pink lines are girls and blue lines are boys. Graphs were generated from the WHO Anthro³⁸³ programme.

Changes in z-scores over time

The z-scores generally increased significantly over time, with the exception of length-for-age which decreased over time (Table 3.7).

Table 3.7 Mean \pm SD changes in z-scores between visits.

	Visit 1 to 4	Visit 1 to 5	Visit 1 to 8	Visit 5 to 8
Weight-for-length, z-score	0.22 \pm 0.44 ^b	0.27 \pm 0.60 ^b	0.60 \pm 0.89 ^c	0.35 \pm 0.68 ^b
Weight-for-age, z-score	0.01 \pm 0.17	0.05 \pm 0.32	0.35 \pm 0.77 ^b	0.32 \pm 0.70 ^b
Length-for-age, z-score	-0.22 \pm 0.48 ^b	-0.21 \pm 0.55 ^a	-0.25 \pm 0.82 ^a	-0.05 \pm 0.84
BMI-for-age, z-score	0.19 \pm 0.17 ^b	0.23 \pm 0.56 ^b	0.68 \pm 0.88 ^c	0.49 \pm 0.71 ^c
Skinfolds				
Triceps-for-age, z-score	0.15 \pm 0.82	0.55 \pm 0.91 ^c	1.10 \pm 0.99 ^c	0.54 \pm 1.34 ^b
Subscapular-for-age, z-score	0.11 \pm 0.98	0.33 \pm 0.95 ^a	1.16 \pm 0.96 ^c	0.86 \pm 1.09 ^c

Difference between visits (paired t-tests); ^a P <0.05, ^b P <0.01, ^c P <0.001.

3.3.7 Intra-observer variation in anthropometric measurements

Results of the calculations of intra-observer variation are shown in Table 3.8 together with the mean of the six values reported from the MGRS. The intra-observer variations in the First-Feed study were significantly higher for all three measurements.

Table 3.8 Mean intra-observer variation of anthropometric measurements for the First-Feed study and the WHO MGRS³⁸⁶.

	First-Feed (n = 12)	MGRS ^a (n = 1185)	P ^b
Length, cm	0.55	0.36	0.019
Subscapular skinfold, mm	0.78	0.34	<0.001
Triceps skinfold, mm	0.80	0.47	<0.001

^a Mean calculated as the total variation of 6 sites divided by 6³⁸⁶.

^b Difference from MGRS (one-sample t-test).

The intra-observer variations found in the First-Feed study were expected to be higher due to less experience and training and a less vigorous study protocol, than the one reported for the MGRS³³⁸. Thus the intra-observer variation found in the present study is important to bear in mind, when interpreting the results of the anthropometric measurements.

3.4 Discussion

3.4.1 Main findings

The First-Feed study had a very high rate of exclusive breast-feeding at the 2nd time-point amongst those who were included (41/50, 82%), because the participants were specifically approached for their motivation to breast-feed as defined by WHO². The sample of participants was affluent with a high maternal education level, high maternal age relative to parity and high prevalence of factors indicating health conscious behaviours (e.g. all were non-smokers). Hence, the present sample of mothers might only be representative of mothers who intend to breast-feed for an extended duration. The mothers' BMIs were, on average, just under the cut-off between healthy and overweight, and nearly half the mothers were overweight or obese at 15 weeks post-partum. During the study there were small but significant improvements in all variables of maternal anthropometry, indicative of loss of weight and fat mass at the group level.

The group of infants were, on average, born of normal weight, and increased significantly in weight, length and knee-heel length, but not in skinfolds, during the study. Relative to the WHO Child Growth standards, the infants had small but significant differences in anthropometric variables. In addition, infant growth relative to the WHO Child Growth Standards indicated that this group of infants increased more in weight-for-length and BMI-for-length, as well as in skinfolds during and after exclusive breast-feeding, while length-for-age decreased slightly during exclusive breast-feeding. However, the results on skinfold measurements should be interpreted with care due to the high intra-observer variation, the possibility of measurement bias and a sample size too small to detect differences in skinfolds during the study.

In summary, there was no indication that the infants in the present study were unusually small and/or growing unusually slowly, which could have resulted in low breast milk energy requirements during exclusive breast-feeding. Thus the Reilly-Wells hypothesis in terms of infant size being unusually small and/or infants growing unusually could not be confirmed in the present study.

3.4.2 Participation and socio-demographic determinants

Inclusion criteria, representativeness and participation

The mothers in the present sample possessed many characteristics positively associated with breast-feeding. This made the present sample unrepresentative of mothers in Scotland, but this was anticipated due to the necessary inclusion criteria of exclusive breast-feeding at the 1st time-point, and the intention to exclusively breast-feed for 6 months. Only mothers that had initiated and sustained exclusive breast-feeding were eligible for the study, thus, when breast-feeding prevalence is strongly socially patterned²⁰⁷, it was precluded to obtain a representative sample. The only possible attempt to avoid such selection bias, was to recruit mother-infant pairs from an area (Greater Glasgow) with a relatively high degree of deprivation and where breast-feeding prevalence is among the lowest in Europe⁸. Despite this, the mothers in the present study comprised an affluent and well-educated sample, as is typical of mothers attempting prolonged exclusive breast-feeding²⁰⁷ in developed countries.

The First-Feed study had a very high rate of exclusive breast-feeding as 82% of the included participants were successful at exclusive breast-feeding at the 2nd time-point (around 6 months). However, another 10 mother-infant pairs were recruited, but not included into the study. Three of those 10 mothers had started supplementing with infant formula between the time of recruitment and the time of inclusion into the study. This probably represents the normal attrition rate amongst mothers in Glasgow. When so many mothers included in the present study managed to persevere with exclusive breast-feeding to around 6 months, it suggests a possible intervention effect from participating in the study. Anecdotally, several mothers expressed interest in knowing just how much milk they were producing for their infant at the 2nd time-point, as a motivational factor for them to persevere with exclusive breast-feeding.

Six mother-infant pairs had introduced complementary foods by the 2nd time-point, but even amongst these infants, intake of complementary foods was only substantial for two infants, while four infants were still predominantly breast-fed. Therefore the feeding groups were too homogenous to detect any differences in socio-demographic or other variables.

Urbanisation and affluence

The participants in the present study were living in urban or suburban areas of Greater Glasgow with only a few exceptions, and urbanisation has been found to be positively associated with breast-feeding²¹². The SIMD decile distribution revealed a wide distribution, but less deprived areas were over-represented. Consistently, studies from the developed world find that women from affluent areas are more likely to breast-feed than women from deprived areas^{12,195,199,389}. This is also true when affluence is measured as family income, which has also shown to be positively associated with breast-feeding initiation, exclusivity and duration^{195,207,390}. In the UK, the council tax valuation band²¹¹ and the IMD¹⁹¹ have both been found to predict breast-feeding prevalence and is also strongly linked to other socio-economic factors (e.g. family income) which are associated with breast-feeding.

Maternal age, educational attainment and parity

The mothers in the present study were characterised by high education levels, high age relative to parity, and were all married or living with partner. It is plausible that the influence of such socio-demographic characteristics on the prevalence of breast-feeding is mediated by health-related behaviours and/or by knowledge of them. For instance, maternal education level and age at childbirth have both been shown consistently to be positively associated with breast-feeding prevalence^{195,199,203,207,218,238,391-393}. One explanation is that both these factors relate to adult maturity and to being better equipped to understand and adopt breast-feeding recommendations, both through increased life experience and through education. Both factors also increase the chance of having knowledge about the health benefits of breastfeeding³⁹³. Maternal age and education level might also be related in the sense that spending more time on further education might result in a higher maternal age at childbirth.

Recent data from the Office of National Statistics reveal the average age of first-time mothers in the UK to be 27.4 years³⁹⁴, whilst the average age in the present study was 33.7 years with 70% of the mothers being primiparous. Data from ISD Scotland suggests that maternal age at childbirth has generally been rising for the past 30 years in Scotland and that age of primiparity increases with decreasing deprivation, so that mothers of less deprived areas tend to have their first child later in life³⁹⁵.

Parity has been associated with breast-feeding prevalence in many studies, but a review of such studies suggested that the direction of this association differs between studies²⁰⁷. The UK Infant Feeding Survey from 2005 found a negative association between parity and breast-feeding prevalence¹⁸², which theoretically could be explained by higher parity increasing the risk of having experienced difficulty when breast-feeding a previous infant. In addition, having older children to care for at the same time as caring for an infant might prevent the mother from devoting the time and resources it takes to establish breast-feeding²⁰⁷. This might especially be the case, if the interval between births is short. However, a positive association between parity and breast-feeding prevalence has also been found²¹², and it is also likely that mothers might experience breast-feeding as being less difficult than bottle-feeding, and therefore will frequently persevere for longer, if they have breast-fed successfully before.

Smoking and maternal BMI

There were no smokers in the First-Feed study. Maternal smoking is a socio-demographic factor that may have a negative impact on maternal lactation physiology. Maternal smoking has been found to be associated with lower prevalence of exclusive breast-feeding at 3 months - 4 months^{212,391,393} and any breast-feeding^{222,225,226,392,393}. A meta-analysis found a higher probability of early weaning before 3 months in smoking mothers than non-smoking mothers³⁹⁶. It should be noted that smoking itself is a health-related behaviour positively associated with deprivation. Smoking is also associated with lower milk volume and milk fat production³⁹⁷, and the suggested mechanism is that nicotine reduces prolactin levels, affecting breast milk production in an adverse way³⁹⁷.

In the present study, maternal BMI was on average just within the healthy BMI range, and there were small significant improvements at the group level in all measurements of maternal body size and composition during the course of the study. Studies from the US^{227,398,399}, France⁴⁰⁰ and Australia^{401,402} have found evidence of lower breast-feeding incidence and prevalence amongst pre-pregnant obese women compared to women of normal pre-pregnant BMI. A Danish study found a dose-response relationship between BMI-category and the risk of early termination of any breast-feeding in a large data set from the Danish National Birth Cohort after adjustment for confounders⁴⁰³.

Suggested mechanisms from animal studies include metabolic disturbances and abnormal steroid hormone levels which may contribute to lactation difficulties⁴⁰². This is in addition to an increased risk of experiencing breast-feeding problems and a poorer ability to address those problems amongst overweight and obese mothers^{398,401}.

3.4.3 Infant size and growth

Overall, the infants in the present study were not unusually small and they did not grow unusually slowly. Hence, in the present sample, infant size and growth could not explain the shortfall in energy supply during exclusive breast-feeding to 6 months, described in section 1.3.1. Therefore, the findings in the present study in terms of infant size and growth were not consistent with the Reilly-Wells hypothesis.

Anthropometry variables

The anthropometric variables revealed infant growth as significant increases in weight, length and knee-heel length during the study. Within the weeks of measurements at the 1st and 2nd time-points, growth was apparent as significant increases in weight. Whilst crown-heel-length did not increase significantly within the weeks of measurements, a significant increase was found for the knee-heel-length at the 1st time-point. The knee-heel-length measurement was added as an extra anthropometric measurement in the present study, because it is very feasible to use in the field setting and validation studies have found good accuracy and precision of the measurement³⁴⁰. The lower leg grows relatively more in length, than the total body, and, expressed as a percentage of the crown-heel-length, it has been found to increase from about 25% of crown-heel-length at birth to about 27% at 12 months of age³⁴². This relatively fast growth during infancy together with the high precision of the measurement makes it possible to detect short term changes in linear growth, where the crown-heel-length measurement is too imprecise.

No significant changes were found in skinfolds during the present study. WHO Child Growth Standard chart percentiles indicate that both triceps and subscapular skinfolds slowly decline during infancy³⁸³, but they may be fairly insensitive to short-term growth in samples of limited size, such as the present study.

In the present study, there were consistent trends for sex differences in body size with boys being bigger than girls, but most of these differences were not significant. There were also no significant differences in growth between sexes. A similar finding was, for instance, found in the study by Butte and colleagues with normative data for infant size and body composition during the first 2 years of life¹⁴⁵. Since the sex differences were relatively small in the present study, they might only have been significant, if the study had included a larger sample of infants. The issue of differences between sexes in milk intake and energy balance will be explored further in Chapters 5 and 6, respectively.

Growth relative to WHO Child Growth Standards

The WHO Child Growth Standards were chosen as a reference for the present study because they are meant to be prescriptive for normal growth of healthy breast-fed infants under optimal conditions³⁷⁵. However, the infants in the present study varied from the WHO Child Growth Standards, evident as several mean z-scores significantly different from zero at the group level, although the magnitude of these differences were small. Furthermore, the results of the present study indicated that the infants showed a different pattern of growth compared to the WHO Child Growth Standards, evident as significant changes in mean z-scores over time in for instance weight-for-length, length-for-age and BMI-for-age, as well as in biceps-for-age and triceps-for age - both during and after exclusive breast-feeding. For instance, the lack of change in the measurement of triceps skinfolds over time coupled with the significant change in triceps-for-age z-scores can have two explanations. One is that the measurements of skinfolds were so imprecise to detect any change over time (section 3.4.4), which they did find in the MGRS with much larger sample sizes and much higher precision of measurements. The other interpretation is that the infants in the present study retain or store sub-cutaneous fat slightly differently than the infants in the MGRS. If this is the case, it could be because the two studies have different degrees of adherence to the recommendation of 6 months exclusive breast-feeding, as defined by WHO². Large studies conducted in Belgium and Norway³⁸⁵ as well as the randomised controlled trial from Iceland⁵⁴ also revealed significant deviations in their distributions of anthropometric variables compared to the WHO Child Growth Standard, identifying more infants beyond $\pm 2SD$ in z-score than when using national growth references³⁸⁵.

Data for the WHO Child Growth Standards were collected from 1997 - 2003³⁷⁵, and it was during this time that the WHO changed their recommendation to exclusive breast-feeding to 6 months¹. The MGRS was therefore designed with the inclusion criteria of exclusive or predominant breast-feeding until 4 months, and two other inclusion criteria stated introduction of complementary foods between 4 and 6 months, and continued partial breast-feeding until 12 months³⁷⁶. The MGRS revealed varying compliance with these three inclusion criteria³⁷⁶. The lowest compliance was Brazil with only 22.3% of mothers compliant with the three feeding criteria, while the highest compliance was Ghana with 69.3%³⁷⁶. Given that feeding mode affects infant growth (see below), this could help explain some of the mean z-scores found to be significantly different from zero in the present study (as well as in other studies), as well as the significant changes in z-scores over time. At the 1st time-point, where the feeding mode should be most similar between the First-Feed study and the MGRS, it is only the skinfold z-scores that are significantly different from zero.

Growth is influenced by feeding mode

Many studies have observed different growth patterns between formula-fed and breast-fed infants^{140-145,404} - although infants were generally not exclusively breast-fed beyond 4 months in these studies. The main difference seems to be a faster growth of breast-fed infants in the first 2 - 3 months of life followed by a slower growth for the rest of the first year of life. These differences in growth between feeding modes may be due to differences in energy and protein intake (section 1.1.3). One study found that formula-fed infants had about 15% higher energy intake and 66% higher protein intake than exclusively breast-fed infants at 3 months of age¹³⁴. Different hormonal responses to feeding modes, including higher insulin levels of formula-fed infants, has also been demonstrated in neonates¹⁰⁶.

It has been suggested that growth is influenced more by the actions of IGF-1, than Growth Hormone during the first two years of life, and IGF-1 release is stimulated by protein and energy intake as well as other nutritional factors⁴⁰⁵. Therefore, growth in the first two years is perhaps more reflective of infant feeding and nutritional status, than later in life, and growth is therefore regarded as a reliable indicator of whether infant energy requirements are met.

The issue of different protein intakes of formula and breast-fed infants has recently been tested in a large double-blind randomised trial, the European Childhood Obesity Project¹²². In this trial, bottle fed infants were randomised to receive either conventional formula or reduced protein formula until 1 year. A control group of infants exclusively breast-fed according to the recommendation of ESPGHAN (section 1.1.2) was included as controls. The result was a clear difference in growth, with infants on the low protein formula having a significantly lower weight-for-age, weight-for-length and BMI-for-age z-score, and their growth pattern were closer to those of the breast-fed controls than the higher protein formula group⁴⁰⁶. Hence, the epidemiological evidence of a relationship between infant feeding and growth has been made plausible through suggested mechanisms of hormonal influence, and subsequently been made probable by the confirmation of the hypothesis in a randomised trial.

Implications of using growth charts

Child growth monitoring is highly dependent on the growth chart used. Studies comparing growth of breast-fed infants have found an increased risk of being identified as growth faltering when using NCHS reference charts³⁸⁴ or the CDC 2000 reference charts⁴⁰⁷ compared to the WHO Child Growth Standards. The WHO Child Growth Standards also produced tighter distributions of growth due to less variance in the data, and therefore more infants would risk being identified as wasted and/or overweight ($\leq 2SD$ and/or $\geq 2SD$ in weight-for-length, respectively) compared to the NCHS reference charts³⁸⁴.

At the time of conducting the present study, the growth charts in the “red book” were based on the UK 1990 growth reference²⁹⁶ generated from infants that were largely formula- or mixed fed. In Scotland the WHO Child Growth Standards were adopted in January 2010, so that all infants born after this date will have health visitor records of infant growth plotted on WHO Child Growth Standard charts in their “red book”.

For exclusively breast-fed infants, the most appropriate growth reference at the present time is probably the WHO Child Growth Standards. However, small significant differences in size and growth were observed in the present study, and similar findings have been revealed from other countries^{385,408,409}.

This raises the concern that using growth charts based on the WHO Child Growth Standards in populations with higher rates of breast-feeding may affect breast-feeding rates negatively, when these infants are not showing growth patterns in concordance with the WHO Child Growth Standards^{385,408}. If an infant should fall below its growth trajectory on a growth chart, the mother may interpret this as growth faltering, leading to an unnecessary intervention⁴¹⁰, e.g. introduction of complementary foods or formula supplementation. This problem has been reported before⁴¹¹. Conversely, if an infant crosses upwards on a growth chart, this may be interpreted by the mother as the infant growing faster in both size and maturity, than “normal”, and may therefore conclude that the infant is ready for complementary foods. Anecdotally, many mothers in the present study took great concern in their infants’ growth, discussing infant weight gain with peers and studying growth charts in the “red book”. Additionally, it seems that parents generally receive very few instructions in how to use and interpret growth charts appropriately⁴¹², which may add to the risk of mothers adopting infant feeding practices that are detrimental to breast-feeding success.

Using a growth reference that reflects appropriate infant growth is important for growth monitoring⁴¹³. The MGRS used to generate the WHO Child Growth Standards had more frequent measurements of higher quality, than any previous reference growth chart, and this makes it more suitable for accommodating the rapid changes in growth velocity that occurs during infancy⁴⁰⁷. Using the WHO Child Growth Standards is therefore considered an improvement over the previous growth charts, although any growth chart will only be representative for contemporary infant growth⁴¹⁴. Using them for all infants in Scotland, including partially breast-fed or formula-fed infants, may help promote the growth of these infants to be more in line with breast-fed infants.

3.4.4 Limitations and strengths of the present study

The sample of mother-infant pairs in the present study was not representative in terms of socio-demographic characteristics. As these characteristics are strongly associated with breast-feeding prevalence, the results in terms of breast-feeding prevalence (and breast-feeding practices as will be discussed in Chapter 7) is not representative either. However, the First-Feed study was designed as a physiological study with the primary aims of exploring the lactational and energetic adaptations to exclusive breast-feeding for 6 months.

As revealed by the evaluation of intra-observer variation in the present study, precision of infant anthropometric measurements were not as good as in the MGRS⁴¹⁵. Therefore, results from skinfold measurement variables in particular, but also length variables, should be interpreted with some caution, and have been used cautiously in the present study. When the skinfold measurements had poor precision - revealed as high intra-observer variation - this will also have affected calculation of skinfold z-scores. From the standardisation sessions in the MGRS it was revealed that the trained anthropometrists tended to underestimate length and overestimate skinfolds relative to the expert⁴¹⁵.

In the present study, skinfold measurements were generally lower than the WHO Child Growth Standards. Therefore, if this were due to measurement bias alone, skinfold measurements would have been underestimated. However, it is possible to explain these results - at least in part - from the theory of the effect of feeding mode on infant growth and body composition. It could have been interesting to explore whether the infants in the present study had a growth between birth and 15 weeks of age, which was closer to that of the infants in the MGRS, as infant feeding during early infancy may have been even more similar between the two studies. However, including measurements between birth and 15 weeks of age would have necessitated more time and a larger research grant than that which was within the means of the First-Feed study.

The intra-observer variation on the length measurement was 0.55 cm. In comparison the mean growth in length between the 1st time-point and the midway visit (about 5 weeks) was 2 cm. Therefore, the imprecision may have prevented the detecting the growth in crown-heel-length during the week of measurement at the 1st time-point, in the same way as it was detected in knee-heel-length.

The scale used for measuring infant body weight was a digital scale with a low risk of intra-observer variation. However, the increment of the weighing scale in the present study was 20g, and therefore the precision of the measurement was probably lower than this²⁶², and this may have affected the measurement of weight gain during the week of measurement - particularly at the 2nd time-point, where growth velocity was lower than at the 1st time-point. However, scales with smaller increments are often less appropriate for the field setting because the transport and movement of the scale may affect their calibration.

The main strength of the present study was the high rate of exclusive breast-feeding to around 6 months of age, as defined by WHO². The design included frequent measurements, using methodologies appropriate for the field setting. With this data, the First-Feed study presents a unique opportunity to explore longitudinal changes in breast milk intake and infant energy balance in exclusively breast-fed infants who were growing normally.

3.5 Summary

The participants in the First-Feed study were highly prevalent in socio-demographic factors positively associated with breast-feeding. They were fairly affluent and not representative of Scottish mothers in general, but this was expected due to the design and inclusion criteria of the present study. The study had a high completion rate amongst the participants, and a very high rate of exclusive breast-feeding to around 6 months, indicating a possible intervention effect from participating in the study.

It was an aim to describe the participants of the First-Feed study in some detail, and the data presented here will allow researchers to compare with other studies performed elsewhere. In the present study, there was no evidence of the infants being unusually small or growing unusually slowly at the group level. Therefore, this chapter could not confirm infants being small or growing unusually slowly as explanations for the shortfall between breast milk energy intake and estimated energy requirements as identified in the Reilly-Wells hypothesis.

CHAPTER 4 EVALUATION OF DOSE ADMINISTRATION TO INFANTS

4.1 Issues of validation

The present chapter reports on the evaluation of the dose-to-infant procedure, and this evaluation was published as part of the papers generated from the First-Feed study¹⁵. In any research project it is important to interpret the data in the light of the methodology used to obtain them. All methodologies have strengths and weaknesses. For instance, methodologies appropriate for field studies may be quick and convenient, but then have issues with precision and/or accuracy. In the previous chapter, infant anthropometry was interpreted in the light of the intra-observer variation that compromised measurement precision. Similarly, before interpreting the data obtained from the DLW method, it was important to explore issues with this methodology, which could affect the results.

4.1.1 Validation of the doubly-labelled water method

The DLW method has been extensively validated in many population groups³²⁵. However, it relies on the participants to cooperate and consume an accurately measured amount of DLW, and such cooperation is not guaranteed, when working with infants. Therefore, as part of the First-Feed study, it was also an objective to evaluate the dose-to-infant procedure.

The DLW method involved five steps and each of these steps should be evaluated to determine if and how they may affect the overall accuracy and precision of the DLW method. The urine sampling procedure (step 2) used for infants has been evaluated, where the risk of isotope sequestration through water evaporation from the nappy was investigated³¹⁶. The urine sample analysis (step 3) is almost fully automated, and the mass spectrometers generally had good analytical precision (section 2.3.4). The equations used to derive the outcome variables (step 4 and 5) have been tested for their assumptions and are generally accepted^{305,310,318,329,416-420} (Appendix B). However, isotopic methods rely on a very accurate and precise dose administration (step 1), but this had not previously been evaluated in exclusively breast-fed infants.

Dose-to-infant administration

For dose administration to formula-fed infants, DLW can be mixed with formula in the infant's feeding bottle, and formula-fed infants can usually drink from the feeding bottle with minimal spills³¹¹. In hospitalised infants, DLW has been administered through an inserted naso-gastric tube (feeding tube) with no spills being encountered^{246,306,318}, but this method is invasive, and therefore unethical to adopt for healthy free-living infants. Predominantly breast-fed infants usually have some prior experience with drinking water^{313,315} and drinking from other sources than the breast. However, for healthy, exclusively breast-fed infants the only option is to administer the dose orally with the infants' co-operation²⁷⁹, and, as they have no prior experience of consuming water, this can pose a challenge to researchers.

Accuracy and precision

Dose spills are a common issue in all stable isotope tracer studies³⁰⁵. In infants this problem is more pronounced when the concentration of isotopes in the dose given is high (dose is given undiluted) and the total volume of dose is small. During dose administration, there is a risk of losing dose through un-quantified spills, and any lost dose not accounted for will result in the dose consumed being overestimated, which will affect the accuracy of the calculated outcome variables. Furthermore, dose spills during dose administration to infants often consists of dribbles from the mouth rather than spills directly from the dosing equipment. Therefore, any dose spills that are accounted for and quantified still impair precision of the method because isotopic enrichment of the spills cannot be ascertained. This is important because a method with poor precision generally requires larger sample sizes to reveal the true results. Assuming that all quantified spills are either dose or saliva would introduce systematic bias in the calculation of the dose consumed. However, either case would not necessarily result in unrealistic values of subsequently calculated outcome variables. The accuracy of the calculation of the dose consumed can therefore not be assessed objectively.

As described in section 2.3.5, TBW is calculated from the dilution spaces of the isotopes, which are also used to calculate other variables (e.g. FFM and $r\text{CO}_2$, section 2.3.5 and 2.3.6). However, the calculation of dilution spaces depend on knowing the exact amount of dose consumed.

If the dose consumed is overestimated due to either un-quantified spills, or by assuming that all quantified spills are saliva when in fact they do contain DLW, the dilution spaces will also be overestimated, and this will then affect the value of TBW. Even though spills are a real issue in the dose-to-infant procedure, there seems to be no literature evaluating this in detail.

4.1.2 Aims of the present chapter

Therefore, the aims of the present chapter were to:

- Account for numbers of successful dose administrations and IRMS analyses, and assess the practical utility of the DLW dose-to-infant procedure as the rate of successful dose administrations.
- Explore the feasibility of different methods of dose administrations.
- Describe quantified dose spills and illustrate the effect of those spills on the calculated TBW.

4.2 Methods

The preparation for the dose administration and the dose-to-infant procedure was described in section 2.3.2. The actual circumstances surrounding the IRMS analyses was reported in section 2.3.4, and the quality criteria for successful IRMS analysis were described in section 2.3.5.

4.2.1 Evaluation of the dose-to-infant procedure

Practical utility of the dose-to-infant procedure

The practical utility of the dose-to-infant procedure in exclusively breast-fed infants was examined by the rate of successful dose administrations. A dose administration was defined as unsuccessful, if infant uncooperativeness resulted in spills not being quantifiable (e.g. dose lost on clothes), or if the infant had large amounts of reflux (too much for it to be contained on the pre-weighed tissues) during or within 20 minutes after the dose administration.

Feasibility of the dose-to-infant procedure

The methods of dose administration used in the present study were syringe, feeding tube, or bottle, as described in section 2.3.2.

The decision as to which of these three methods to use was taken in collaboration with the mother. For some infants, more than one method was tried, to improve infant tolerance to the dose administration. Other potential methods of administration could be spoon or cup feeding, which were abandoned when planning the present study as it was deemed more difficult to ensure that all spills were collected when using these methods. For the present evaluation, the infants were categorised according to the method of administration (syringe, feeding tube, or feeding bottle) which resulted in best possible tolerance to the procedure. The duration of dose administration was quantified to the nearest 5 minutes, and this often included breaks for feeding, nursing or if the infant needed a nap.

Quantifying dose spills

Spills were quantified through pre- and post-weighing of dosing equipment and tissues, as described in section 2.3.2. The observed viscosity and likely composition of the spills (particularly if it contained residues of milk etc.) was noted down at each dose administration (Appendix A.7), and for the majority of spills, which were characterised as dribbles, it was assumed to be half dose and half saliva. This was used as a correction factor in the calculation of the amount of dose consumed. For example, if pre- and post- weighing of the dosing equipment revealed an amount of DLW given as 20.117g and the amount of spills on tissues (from pre- and post- weighing) was 0.987g, noted as dribbles, then the dose consumed was calculated as $20.117 - (0.5 \times 0.987) = 19.624\text{g}$.

4.2.2 Statistical methods

Data on the practical utility, feasibility and dose spills were summarised as described in section 2.7.2. The spills were expressed as percentage of the amount of dose prepared. The elimination spreadsheets were evaluated according to the quality criteria and TBW was calculated from isotope dilution spaces, as described in section 2.3.5. In order to illustrate the impact of different approaches to the calculation of dose consumed, TBW was calculated 1) assuming all spills were dose, 2) correcting for the composition of spills, and 3) assuming all spills were saliva.

4.3 Results

4.3.1. Practical utility of the dose-to-infant procedure

Out of the 94 dose administrations, seven (7.4%) were unsuccessful by the definition in section 4.2.1 (Figure 4.1). Three dose administrations were unsuccessful due to large amounts of regurgitation and four were unsuccessful due to uncooperativeness, which led to the infant spitting out an unquantifiable amount of dose. A further 13 measurements did not comply with our quality criteria for the elimination spreadsheets, as described in section 2.3.5.

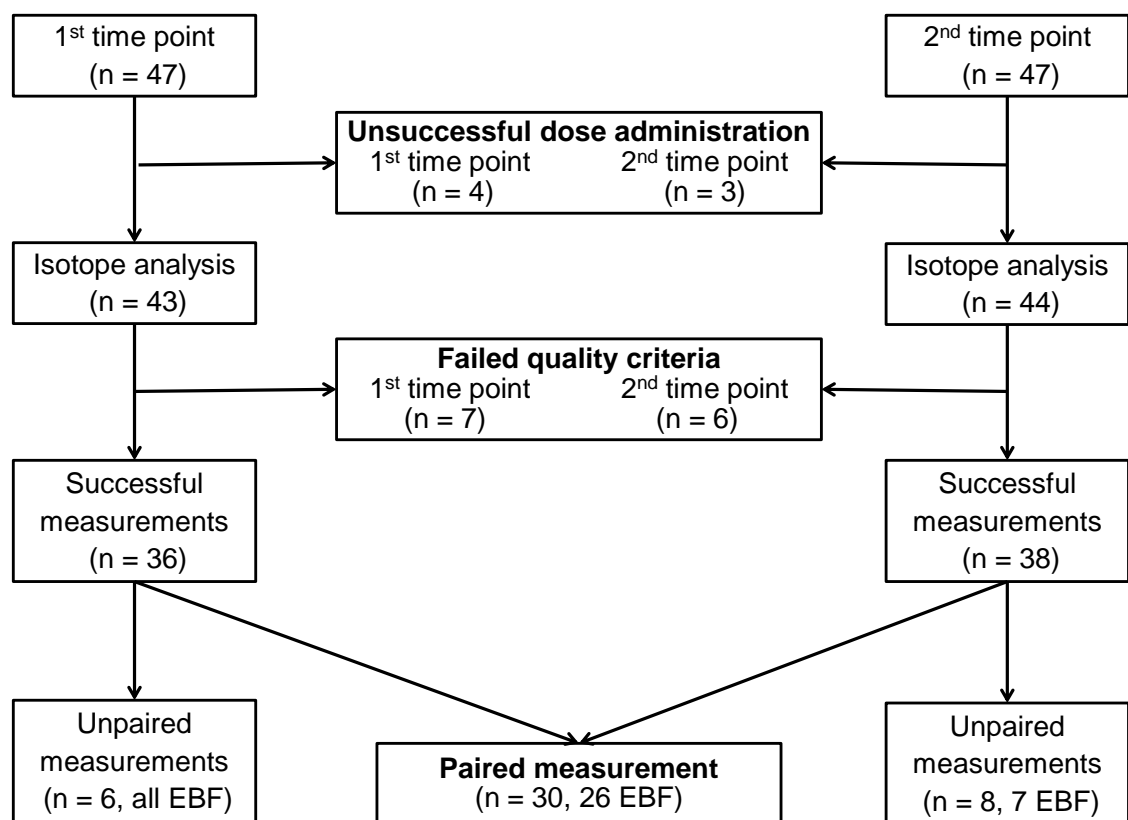


Figure 4.1 Overview of measurements in terms of dose administrations and isotope analysis

^a Unsuccessful dose administration: Dose could not be given due to infant uncooperativeness or infant had reflux and an unquantifiable amount of doubly-labeled water was lost.

^b Unsuccessful isotope analysis: Quality criteria of isotope space ratio within 1.010 and 1.090 and estimated error on total energy expenditure <10% were not fulfilled.

EBF: Exclusively breast-fed

This resulted in 36 successful measurements at the 1st time-point and 38 successful measurements at the 2nd time-point with 30 paired measurements. Four of these were complementary breast-fed at the 2nd time-point, leaving 26 paired measurements of exclusively breast-fed infants, as defined by WHO².

4.3.2 Feasibility of the dose-to-infant procedure

Overall, the dose administration lasted from 5 to 90 minutes (Table 4.1). The median duration ranged from 10 minutes (using feeding bottle) to 25 minutes (using syringe) at the 1st time-point, and from 20 minutes (using feeding bottle) to 43 minutes (using feeding tube) at the 2nd time-point.

In 23 of the 87 successful dose administrations (26.4%), more than one method of administration was used, in order to improve infant compliance with the dose-to-infant procedure. Using a syringe seemed to result in best tolerance from the infants at both time-points (23/43 and 36/44 dose administrations at the 1st and 2nd time-points, respectively). Using the feeding tube during a feed seemed to be more successful at the 1st time-point than at the 2nd time-point.

Table 4.1 Dosing variables (median (min – max)) for 1st and 2nd time-point.

	n	Prepared dose (g)	Consumed dose ^a (g)	Spills ^b (%)	Dosing time (min)
1st time point	47				
Unsuccessful	4				
Syringe	23	17.9 (12.5 - 23.2)	16.7 (10.9 - 21.2)	5.0 (0.6 - 36.2)	25 (10 - 60)
Feeding tube	12	18.1 (13.8 - 22.7)	17.3 (11.8 - 21.0)	4.8 (0.2 - 20.5)	20 (15 - 35)
Bottle	8	18.5 (13.9 - 22.2)	17.6 (13.9 - 20.6)	6.8 (0.1 - 57.0)	10 (5 - 45)
2nd time point	47				
Unsuccessful	3				
Syringe	36	21.5 (14.7 - 29.1)	19.7 (12.3 - 26.1)	5.3 (1.4 - 32.7)	30 (10 - 90)
Feeding tube	2	20.5 (19.9 - 21.1)	19.6 (19.0 - 20.1)	4.5 (4.3 - 4.7)	43 (25 - 60) ^c
Bottle	6	18.3 (15.0 - 26.7)	15.6 (11.7 - 23.4)	6.6 (2.3 - 11.4)	20 (5 - 25)

^a Consumed dose corrected for spills.

^b Spills presented as percentage of dose prepared.

^c Mean value as two data points precluded a median.

At the 1st time-point, 32 mothers (68%) declined the option of using a feeding bottle during dose administration - either for personal reasons or because of previous experience with the infant refusing to bottle-feed expressed breast milk. At the 2nd time-point, one of these mothers opted to try the bottle, whilst an additional four mothers declined the option (in total 35 mothers declined using the bottle at the 2nd time-point). This collaboration between the mother and the researcher regarding method of dose administration precluded any random allocation of the method of administration, and therefore also any formal testing of which method was most feasible.

4.3.3 Dose spills and their effect on calculated total body water

Figure 4.2 presents the distribution of spills from dose administrations. Spills ranged from 0.15 - 56.95% of the dose prepared, but the majority (63 of dose administrations, 72%) had spills of <10%. None of the three methods of administration seemed superior in reducing spills, though the study was underpowered to test this issue. The percentage of spills correlated weakly with duration of dose administration (Spearman's rank correlation; $r = 0.303$, $P = 0.005$), which may indicate that spills were more dependent on infant tolerance to dose administration or the DLW itself, rather than to the method of administration.

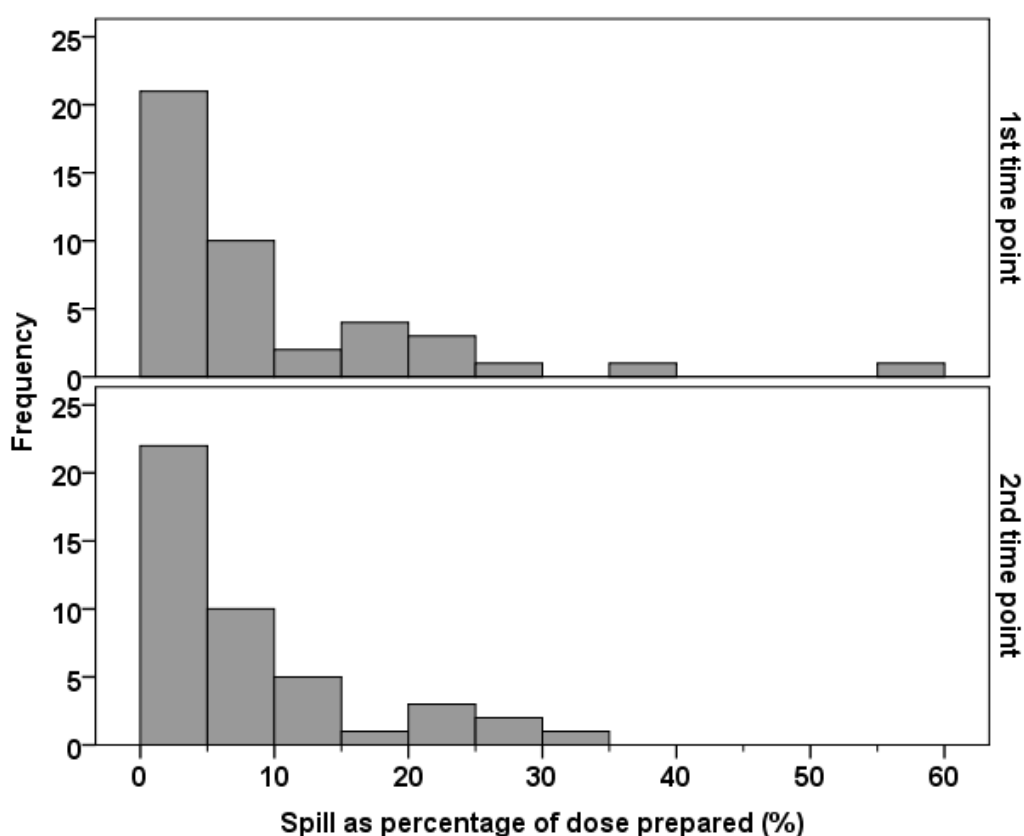


Figure 4.2 Histograms of spills as percentage of the dose prepared for each time-point.

When regarded at group level, the three different approaches to calculation of dose consumed had little impact on the calculated TBW (Figure 4.3). However, at the individual level, the effect on the calculation of TBW would depend on the amount of spills quantified.

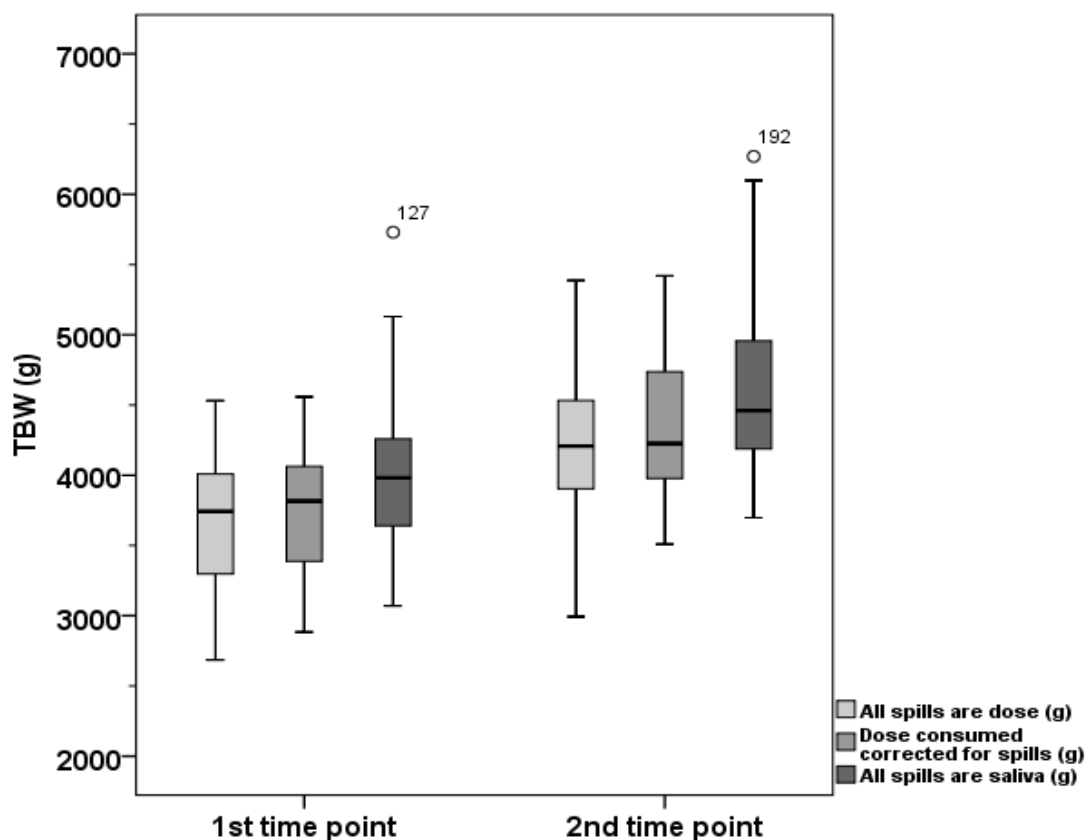


Figure 4.3 Box-plot showing TBW as calculated, when dose consumed are calculated using three approaches.

4.4 Discussion

4.4.1 Main findings

Seven measurements were lost due to unsuccessful dose administrations, thus the practical utility of the dose-to-infant procedure for dose administration of DLW resulted in a success rate of 92.6%, which was deemed good. By allowing time and flexibility in the method of administration (feeding tube, syringe or bottle) the dose-to-infant approach was found to be feasible for exclusively breast-fed infants¹⁵, although the risk of spills was an issue to take into consideration when evaluating the results of the present study.

4.4.2 Approaches to the dose-to-infant procedure

Previous studies using the dose-to-infant procedure on breast-fed or formula-fed infants have reported on dose administration only very briefly. Some studies have used approaches such as dripping the DLW into the infant's mouth using a syringe^{14,314}, a Pasteur pipette³⁶⁴, or a naso-gastric tube^{313,315} (feeding tube). Other studies have reported using a mixture of methods but have not described these in much detail^{311,312}.

In the present study, using the feeding tube for dose administration during a feed was more successful at the 1st time-point than at the 2nd time-point, probably due to the infant being less aware of the presence of the feeding tube. Therefore, this method could be applied for exclusively breast-fed infants aged 4 months or younger - especially if the infant has a good latch onto the breast. In such cases, it was possible to place the feeding tube along the infant's palate and let the negative pressure in the infant's mouth determine the flow of DLW.

The use of a feeding tube as the method of administration has been described before in older infants and infants that were not exclusively breast-fed, but in those studies the feeding tube was used to drip the water into the back of the mouth, or it was attached to the mother's finger for the infant to suck on this while consuming the water^{313,315}. This approach was tried in one pilot session on one infant before the conduct of the present study, but was abandoned as it was difficult to control spills, when using the mother's thumb, and the infant had problems with swallowing the water when the feeding tube was placed at the back of the mouth.

In the present study, the most successful route of administration at the 2nd time-point was the syringe, where the researcher had the best control of the flow of DLW given. However, the syringe was less well tolerated at the 1st time-point, perhaps because it was more difficult for younger infants to swallow the DLW when it was administered this way. The syringe has been used successfully in other studies on infants who were not exclusively breast-fed¹⁴. The procedure is similar to when giving infants medicine (e.g. paracetamol mixtures) or supplements (vitamin supplements or agents to help problems with reflux), and therefore some exclusively breast-fed infants may have some prior experience with this method of dose administration.

4.4.3 Inaccuracy and imprecision caused by spills

The issue of minimising spills and quantifying them precisely when using isotopic techniques on infants is a practical challenge, which has received little attention in the literature³⁰⁵. In the present study, having the infants lying down or in a semi-supine position during dose administration made it easily manageable to collect the spills on most occasions (only four dose administrations were unsuccessful because of spills not accounted for). Furthermore, over the duration of the dose administration, some of the spills caught on tissues could be evaporating. Therefore, great care was taken to change tissues when spills had occurred, and to keep the wet tissues in re-sealable bags to minimise the risk of evaporation.

Butte and colleagues reported that dose losses did not exceed 0.3 g (measured on pre-weighed tissues) in a cross-sectional study on predominantly breast-fed infants at 4 or 6 months of age³¹⁴. In a study on 8 month-old infants using a nasogastric tube it was reported that on average 84% of the dose prepared for the infant had been consumed, but it was unclear from the study, whether this reflected that 16% of the dose prepared were spilled or if it was just not administered to the infant³¹³. The spills in the present study seem to be somewhere in between, and may be deemed acceptable for the purpose of calculating TEE at the group level. In future studies, the effect of spills on the precision of the technique could be overcome by applying a cut-off of 5% or 10% spill as percentage of dose prepared, bearing in mind that this would reduce study power.

The composition of spills was also an issue, as this affected the calculated dose consumed. Assuming that all spills were either dose or saliva would introduce systematic bias in the calculated dose consumed. However, spills in the order of 5% cause a similar error on the measurement of TBW, and hence using either assumption would not necessarily result in unrealistic values of TBW. Therefore, there is no objective way of assessing the accuracy of the calculation of the dose consumed. The size of the bias introduced by assuming all spills to be either dose or saliva would be dependent on the amount of spills tolerated, and could therefore be quite considerable at the individual level, if large spills were accepted.

4.4.4 Limitations and strengths of the present study

Loss of data-points

Unfortunately, the First-Feed study lost 13 measurements due to the elimination spreadsheets not fulfilling the quality criteria (i.e. had an un-physiological space ratio, or an error >10%), which was an indication of analytical error. Reasons for these errors could for instance be DLW dosing administration errors (spills, lost dose), contamination of urine samples (particular the pre-dose samples) and errors in the urine sample time recorded by the mothers. Including all data-points with a successful dose administration may not have changed the findings presented in the following chapters, but would have increased the sample size and study power of the present study. Other studies have included such measurements²⁴², but for the present study it was decided not to include them as the source of the errors could not be identified. Even for measurements which fulfilled our quality criteria, the risk of analytical error affecting the results was still present. However, in spite of our large loss of measurements, but due to the high success rate of exclusive breast-feeding at the 2nd time-point, the sample size was still sufficient according to the power calculation (section 2.7.1), to address the aims of the First-Feed study. This issue will be further addressed in Chapter 5 in relation to milk intake and in Chapter 6 relating to energy intake and energy requirements.

Inaccuracy and imprecision caused by spills

As addressed in the present chapter, the dose-to-infant procedure incurs the risk of spills during dose administration. Although it seemed easy to be confident that all spills were caught on tissues during the dose administrations, any unknown spills that were not quantified or accounted for would affect the accuracy of the present study. Additionally, the spills that were quantified increased the imprecision of the DLW method. We found spills to be very variable, and even though they were <10% for the majority of the dose administrations, this is still a considerable error for the purpose of measuring milk intake and TEE in the present study. The imprecision of spills could have been avoided using deuterium dose-to-mother for measuring milk intake^{28,267}, but this method does not provide as much simultaneous objective data on infant energy balance.

The imprecision caused by spills is additional to the imprecision of the DLW method itself, as the validation studies in infants were in hospital settings where no spills were encountered^{246,306,318}. The imprecision of the DLW method further includes analytical error, biological variation, and errors caused by using mean values of correction factors in the calculation of outcome variables³²⁵ (sections 2.3.5 and 2.3.6 and Appendix B).

Finally, the design of the present study, and the necessity of collaborating with the mother with regards to the dose-to-infant procedure, precluded any randomisation or even systematic testing of the methods of administration available. Therefore, the present study can not be taken as a validation of the dose-to-infant procedure, but the experiences gathered here may help to inform researchers using this methodology in the future. Overall, it seemed that the choice of method of administration for the dose-to-infant procedure to achieve a minimal amount of spills may vary depending on the setting and the infant.

4.5 Summary

The present study found that the DLW dose-to-infant procedure is practical, and allowing sufficient time and flexibility in the method of administration is helpful for its feasibility for use in exclusively breast-fed infants. Spills can be taken into account in the calculation of TBW and other outcome variables by performing a correction that may reduce spills from a bias to an imprecision, but it is important for the precision of the dose-to-infant method to reduce spills as much as possible.

CHAPTER 5 LACTATION PERFORMANCE

5.1 Breast milk intake and milk energy content

The present chapter reports on the main findings of the First-Feed study. The results from this chapter were published as part of one of the papers generated from the First-Feed study¹⁶. In the introduction (sections 1.2.4 - 1.2.5), the evidence collected from two important reviews^{3,42,270} revealed a milk intake from breast-fed infants in the order of 700 - 900 g/d between the ages of 3 and 6 months. Typical milk energy content was found to be about 2.6 kJ/g³. However, the majority of studies included both exclusively and predominantly breast-fed infants^{42,270} and/or used the test-weighing method to measure milk intake³. While cross-sectional data across age-groups in some cases indicated a trend of increasing milk intake over time, this was not confirmed in longitudinal studies, but the longitudinal studies did not follow infants to 6 months of age^{3,42}. Therefore, at the time at which the First-Feed study was designed, there was a paucity of evidence of milk intake in exclusively breast-fed infants, as defined by WHO², to 6 months of age.

5.1.1 The Reilly-Wells hypothesis on milk intake

According to the Reilly-Wells hypothesis, milk intake would have to be unusually high compared to literature values - around 1000 g/d - during exclusive breast-feeding to fulfil infant energy requirements at 6 months of age⁴, based on mean references for energy requirements⁷ and typical infant weight according to the UK 1990 growth reference²⁹⁶. Furthermore, milk intake would have to be increasing over time to accommodate increasing energy requirements.

Other possibilities were that either milk energy content or metabolisability increased significantly over time. If milk energy content was rising, it would be possible to accommodate increasing energy needs while the volume of gross milk intake was fairly stable. From milk samples, gross milk energy content is either measured²⁴¹ or estimated³³⁵ (section 1.2.3) and then converted to metabolisable milk energy content using a factor of metabolisability³³⁶, which is assumed to be the same for all ages.

If metabolisability in reality increases, it would not be evident from using this method. However, the DLW method can be adopted to provide metabolisable milk energy content²⁷⁹ and therefore includes any increase in metabolisability. Further to testing the main hypotheses of the First-Feed study, these possibilities will also be discussed in the present chapter.

5.1.2 Aims of the present chapter

The aims of the present chapter were to:

- Measure metabolisable milk intake at two time-points during exclusive breast-feeding, and compare with the values obtained in the systematic review by Reilly and colleagues³.
- Use paired measurements of milk intake at the two time-points to test if milk intake changes over time in exclusively breast-fed infants.
- Estimate milk energy content and explore changes over time.

5.2 Methods

5.2.1 Data-handling and calculations

The DLW dose-to-infant method was performed as described in section 2.3 and evaluated in Chapter 4. The data-handling of isotope data and the calculations of milk intake and milk energy content were described in section 2.3.5. In brief, the elimination rate of deuterium was used to calculate amount of milk intake²⁷³. The deuterium elimination rate was determined from the multi-point back-extrapolation method²⁵⁵, using the pre-coded spreadsheet¹⁴. Milk energy content (kJ/g) was calculated as TEI (kJ/d) divided by milk intake (g/d), where TEI was calculated as TEE plus E_{growth} . TEI, TEE and E_{growth} will be presented and discussed in Chapter 6.

5.2.2 Statistical methods

The primary outcome variable for the present thesis was the amount of milk intake, and the power calculation revealed that 28 infants with successful repeated measurements would be more than sufficient to detect a significant change between time-points in milk intake (section 2.7.1).

Outcome variables were summarised using descriptive statistics, as stated in section 2.7.2. The amount of milk intake at each time-point was compared to the literature values obtained in the systematic review by Reilly and colleagues³, using one-sample t-tests, to test the null hypothesis of milk intake being similar to literature values at each time-point. The change in amount of milk intake between time-points was assessed with a paired t-test, to test the null hypothesis of no significant change in milk intake over time. In addition, to adjust for the variation in time duration between time-points, a weekly change in milk intake was calculated (assuming a linear increase in milk intake), which was then tested for difference from zero using a one-sample t-test.

Similarly, the secondary outcome variable of milk energy content was tested against the literature value from the systematic review³ using a one-sample t-test, and changes between time-points was examined using a paired t-test. Data-driven tests for differences between sexes (e.g. expecting the larger boys to have higher milk intake than the smaller girls) in both primary and secondary outcome variables were explored using independent t-tests, and Pearson correlations between milk intake and infant characteristics (from Chapter 3) were performed to explore relationships.

5.3 Results

5.3.1 Milk intake

Variables of mean daily milk intake and milk energy content are presented in Table 5.1 with the 2nd time-point presented both for all infants with successful measurements (n = 38) and for exclusively breast-fed infants only (n = 33).

Milk intake compared to literature values

At the 1st time-point, mean \pm SD milk intake was 923 \pm 122 g/d, which was significantly higher than the value of 779 g/d at 3 - 4 months³ (mean difference 144 g/d, 95% CI; 103 to 186 g/d, $P < 0.0001$). At the 2nd time-point, milk intake was 999 \pm 146 g/d for exclusively breast-fed infants, which was significantly higher than the value of 894 g/d at 6 months³ (mean difference 103 g/d 95% CI 56 to 150, $P < 0.0001$). The mean \pm SD milk intake for all infants (n = 38) at the 2nd time-point was 997 \pm 142 g/d.

Table 5.1 Variables of milk intake and milk energy content of all infants and exclusively breast-fed infants only, presented as mean \pm SD.

	1 st time point, All infants		2 nd time point, All infants		Change between time points, All infants		2 nd time point, EBF infants		Change between time points, EBF infants	
	Mean \pm SD	n	Mean \pm SD	n	Mean \pm SD	n	Mean \pm SD	n	Mean \pm SD	n
<i>Milk intake (g/d)</i>										
Boys	949 \pm 96	17	1055 \pm 118 ^b	18	108 \pm 81 ^{c,d}	15	1050 \pm 125	16	96 \pm 81 ^e	13
Girls	901 \pm 140	19	945 \pm 144	20	23 \pm 97	15	952 \pm 153	17	27 \pm 95	13
All	923 \pm 122	36	997 \pm 142	38	65 \pm 98 ^f	30	999 \pm 146	33	61 \pm 93 ^g	26
<i>Milk intake (g/kg*d)^a</i>										
Boys	140 \pm 12	17	134 \pm 13	18	-5 \pm 10 ^h	15	134 \pm 14	16	-5 \pm 10	13
Girls	141 \pm 20	19	128 \pm 16	20	-15 \pm 13 ⁱ	15	128 \pm 15	17	-14 \pm 14 ^j	13
All	140 \pm 18	36	130 \pm 15	38	-10 \pm 12 ^k	30	131 \pm 15	33	-10 \pm 12 ^l	26
<i>Milk energy content (kJ/g)</i>										
Boys	2.74 \pm 0.38	17	2.61 \pm 0.38	18	-0.12 \pm 0.54	15	2.60 \pm 0.40	16	-0.12 \pm 0.59	13
Girls	2.71 \pm 0.39	19	2.63 \pm 0.43	20	-0.05 \pm 0.36	15	2.60 \pm 0.46	17	-0.11 \pm 0.36	13
All	2.72 \pm 0.38	36	2.62 \pm 0.40	38	-0.09 \pm 0.45	30	2.60 \pm 0.43	33	-0.12 \pm 0.48	26

All variables were normally distributed, except change in milk energy content between time-points (Shapiro-Wilk's test; $P = 0.012$).

However, upon visual inspection of histogram and normality curve this was considered to be a random finding.

^a Milk intake per kg body weight calculated from mean body weight over the week of measurement.

Differences in milk intake (g/d) between time-points (paired t-tests): ^c all boys, $P < 0.001$; ^e EBF boys, $P = 0.001$; ^f all infants, $P = 0.001$; ^g EBF infants, $P = 0.003$.

Differences in milk intake (g/kg*d) between time-points (paired t-tests): ⁱ all girls, $P = 0.001$, ^j EBF girls, $P = 0.003$, ^k all infants, $P < 0.001$, ^l EBF infants, $P = 0.001$.

Differences in milk intake (g/d) between sexes (independent t-tests): ^b all infants, $P = 0.015$, ^d all infants, $P = 0.015$, ^h all infants, $P = 0.040$

EBF: Exclusively breast-fed

Change in milk intake over time

The median (min - max) interval between the 1st and 2nd time-points was 9.2 (4 - 12) weeks for those with successful paired measurements (n = 30), and during this period the daily milk intake increased by 65 g/d (95% CI 29 to 102 g/d, P = 0.001) for all infants with paired measurements (n = 30) and 61 g/d (95% CI 23 to 99 g/d, P = 0.003) for exclusively breast-fed infants (n = 26). The mean \pm SD weekly increase in milk intake, adjusted for variation in duration between time-points, was 6.4 g/week (95% CI 2.3 to 10.5; P = 0.003) for all infants. Milk intake per kg body weight decreased significantly between time-points both for all and for exclusively breast-fed infants (for both; mean difference -10 g/kg*d, 95% CI -15 to -5 g/kg*d, P <0.001).

Differences between the sexes

Differences between sexes in milk intake are presented in Figures 5.1 and 5.2 below. Boys tended to have higher milk intakes than girls. This was statistically significant at the 2nd time-point for all infants (mean difference 110 g/d, 95% CI 23 to 197 g/d, P = 0.015), and almost statistically significant for the exclusively breast-fed infants (mean difference 98 g/d, 95% CI -1 to 198, P = 0.051).

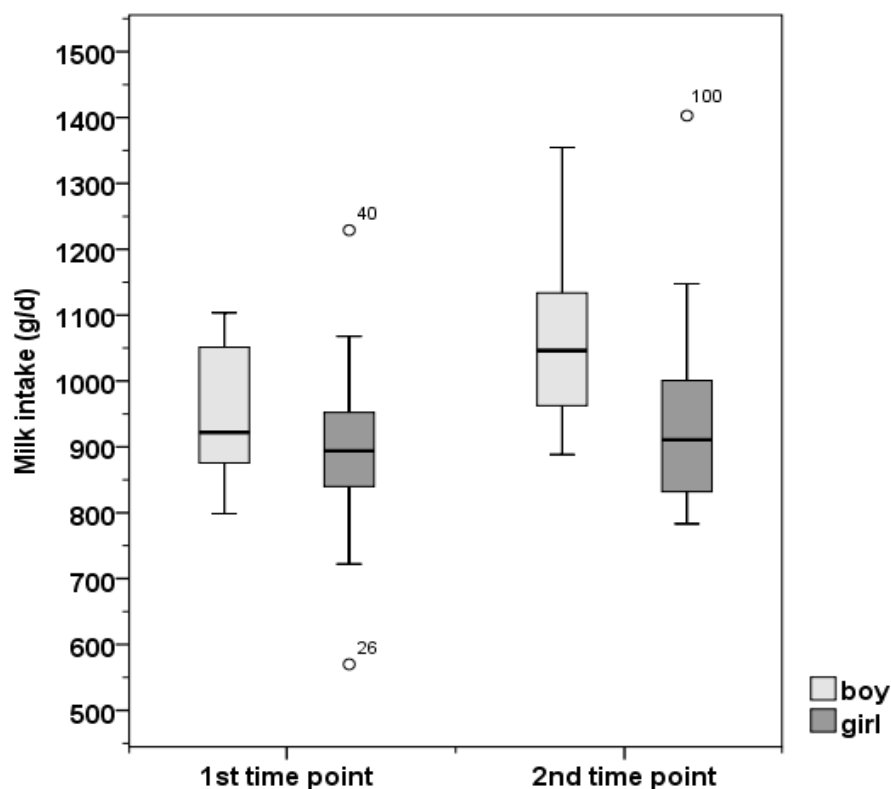


Figure 5.1 Milk intake in g/d for all boys and girls at the 1st (17 boys, 19 girls) and the 2nd (18 boys, 20 girls) time-points.

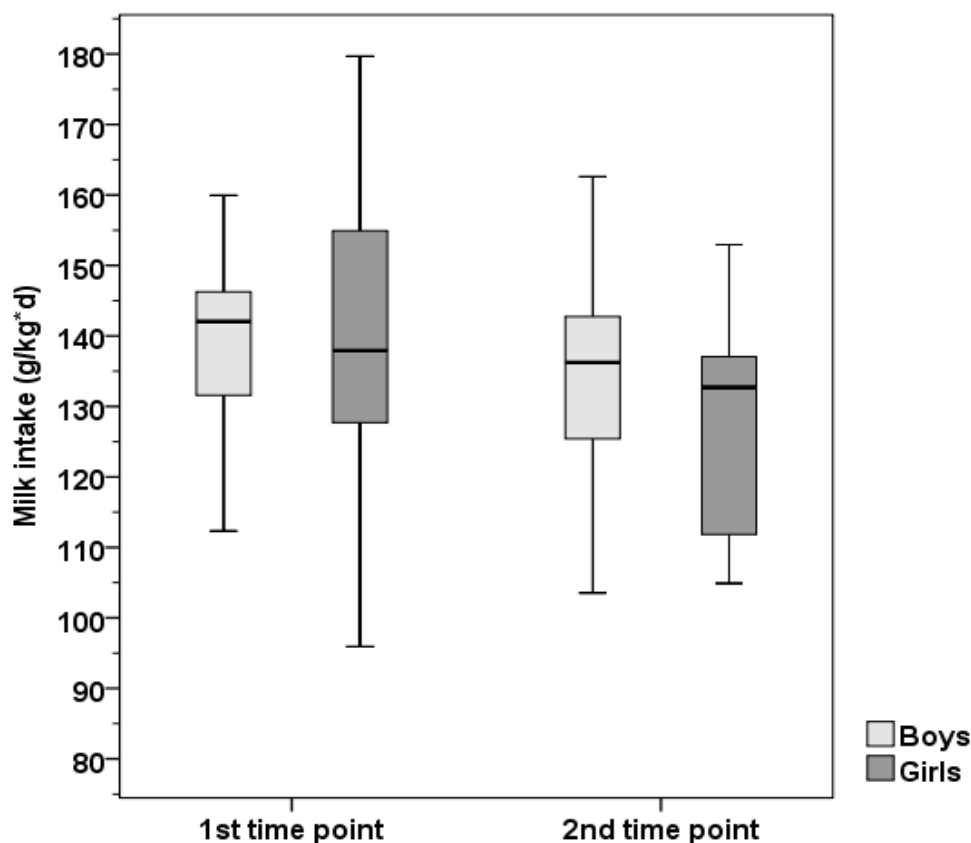


Figure 5.2 Milk intake in g/kg*d for all boys and girls at the 1st (17 boys, 19 girls) and the 2nd (18 boys, 20 girls) time-points.

The boys had a higher increase in milk intake between time-points, and this was significant for boys (all boys; mean difference 108 g/d, 95% CI 63 to 153, $P = 0.015$, exclusively breast-fed boys; mean difference 96 g/d, 95% CI 47 to 144, $P = 0.001$), but not for girls (all girls; mean difference 23 g/d, 95% CI -30 to 77, $P = 0.366$, exclusively breast-fed girls; mean difference 27 g/d, 95% CI -31 to 84, $P = 0.334$). A post hoc power calculation (Cohen's $d = 0.51$) revealed that a sample size of 98 girls would be needed to identify this change in milk intake between time-points as significant.

The decrease in milk intake per kg body weight between time-points was significant for girls (all girls; mean difference -14.7 g/kg*d, 95% CI -22.1 to -7.3, $P = 0.001$, exclusively breast-fed girls; mean difference -13.8 g/kg*d, 95% CI -22.0 to -5.6, $P = 0.003$). For the boys, the decrease in milk intake per kg body weight was much smaller and only significant, when all boys were included (all boys; mean difference -5 g/kg*d, 95% CI -11.3 to 0.4, $P = 0.040$, exclusively breast-fed boys; mean difference -5 g/kg*d, 95% CI -10.9 to 0.0, $P = 0.051$).

5.3.2 Milk energy content

Metabolisable milk energy content was tested against the literature value of 2.6 kJ/g found in the systematic review by Reilly and colleagues³, where milk energy content was mainly determined as gross values, which were corrected for metabolisability³. Neither at each time-point, nor when data from the two time-points were stacked (merged into one variable), were there any significant differences from this mean value (all $P > 0.05$). Milk energy content did not change significantly between time-points, and there were no significant differences between sexes.

5.3.3 Relationships with infant age and size

When data from both time-points were stacked (merged into one variable), milk intake (g/d and g/kg*d) correlated weakly with infant age (not normally distributed) and it correlated moderately with length, knee-heel-length and weight (Table 5.2). For weight gain there was only a correlation with milk intake in g/d, while sum of skinfolds only correlated with milk intake in g/kg*d.

Table 5.2 Correlations between variables of milk intake and infant size^a.

	Milk Intake in g/d		Milk intake in g/kg*d	
	r	P	r	P
Age, wks	0.24	0.040	-0.28	0.017
Crown-heel-length, cm	0.43	<0.001	-0.32	0.006
Knee-heel-length, cm	0.50	<0.001	-0.26	0.023
Mean weight, kg ^b	0.61	<0.001	-0.38	0.001
Weight-gain, g ^c	0.31	0.008	0.16	0.176
Sum of skinfolds, mm ^d	0.20	0.096	-0.32	0.005

^a Correlations performed when data from the 1st and 2nd time-points were stacked (n = 74).

^b Mean weight calculated as a mean of the weights at day 0 and day 7 of each time-point.

^c Weight gain as the difference between the weights on day 0 and day 7 of each time-point.

^d Sum of triceps and subscapular skinfold measurements.

None of the variables of infant age or size above correlated with daily change in milk intake or time-point with milk energy content (data not shown).

5.4 Discussion

5.4.1 Main findings

The present chapter aimed to test the null hypotheses 1) that milk intake would be similar to literature values and 2) milk intake would not change significantly over time after 3 - 4 months of age. The present study found an infant milk intake, which was significantly higher than previously reported literature values³, and which increased significantly over time beyond 3 - 4 months of age at the group level. These findings could not confirm the null-hypotheses, and therefore suggests that the Reilly-Wells hypothesis is supported with regards to milk intake during exclusive breast-feeding. It thus supports the view that exclusive breast-feeding to 6 months is not constrained by maternal physiology⁴²¹⁻⁴²³ and that mothers can accommodate exclusive breast-feeding to 6 months by high and increasing milk outputs - an adaptation not previously described in the literature³. However, the present study found differences in milk intake between the sexes, where boys had about 5% higher daily milk intake at the 1st time-point, than girls, which increased significantly over time (to 10% higher milk intake for exclusively breast-fed boys at the 2nd time-point), while the girls' daily milk intake did not increase. Likewise, milk intake per kg body weight decreased significantly in girls, and this decrease was significantly higher for girls than boys. Finally, the correlation between milk intake and age was weak, whilst the correlations between infant body size and milk intake were stronger.

5.4.2 Differences in milk intake by methodology

In the present study, daily milk intakes at each time-point were much higher than previously published literature values from the developed world³ by around 16% and 11% at the 1st and 2nd time-points, respectively. A possible explanation for these differences in findings could be the use of different methodologies. The literature values were largely based on studies that used the test-weighing method to estimate breast milk intake, while the present study used an isotopic method. Lower estimates of milk intake when using the test-weighing method compared to an isotopic method have been reported before^{3,13,14,268,271,272} (section 1.2.3).

Reilly and colleagues found a mean difference between methods of 66 g/d (95% CI; 11 to 123 g/d, $P = 0.02$), when comparing 38 test-weighing studies with 3 isotopic studies³, with higher values from the isotopic studies, even when the test-weighing studies were corrected for IWL³⁰⁶ and metabolisability³³⁶. The recent study by Da Costa and colleagues²⁷⁰, with pooled data on milk intake obtained by the deuterium dose-to-mother method, also found values of milk intakes at 3 - 4 months that were somewhat higher than those found from mainly test-weighing studies in the systematic review by Reilly and colleagues³, although not as high as the values reported in the present study. It is also worth noting that a well-conducted cross-sectional study in 12-week old infants using the DLW dose-to-infant method also found a relatively high milk intake of 905 g/d⁴²⁰. It thus appears that the DLW dose-to-infant method could be providing slightly higher results of milk intake than the deuterium dose-to-mother method.

It has been suggested, that the test-weighing method is prone to imprecision, particularly in situations of frequent feeding, where the amount of milk consumed at each feed is small compared to the sensitivity of the scale²⁶². Savenije and Brand studied 94 newborn infants, where the person performing the test-weighing was blinded to the amount of milk intake given by the nurse and vice versa²⁶². Milk intake was measured by test-weighing both the infant feeding bottle and the infant on digital scales with an increment of 1 g. These were calibrated and examined for precision, using standard weights. Whilst the accuracy was good at the group level, the precision was poor as the difference between weights (weights of bottle versus weights of infant) ranged from about -30 to 23 ml at a median milk intake of 37.5 ml per feed²⁶². The authors pointed out, that even accurate scales with increments of only 1 g are inappropriate for the test-weighing method, as the imprecision of the scale is much greater²⁶² than the increment.

There have also been concerns that the test-weighing method might be prone to under-reporting¹⁴. For the test-weighing procedure, the mother has to follow a carefully defined procedure of weighing the baby before and after each feed (typically for 24-48 hours), and recording both infant weights as well as start and end times of every feed²⁷¹. This incurs risk of under-reporting due to feeds being missed. Additionally, there can be problems with defining the start and the end of each feed, due to the nature of breast-feeding involving periods of actual feeding mixed with periods of non-nutritive suckling⁴²⁴.

It also entails a risk of obtaining an unrepresentative estimate of milk intake due to day-to-day variability in milk intake, which increases imprecision when the measurement period is short²⁸⁸. Finally, the test-weighing method is intrusive on the breast-feeding routine, and may therefore alter behaviour in a way that influences milk intake.

As noted in section 1.2.3, the isotopic methods do not require the mothers to record any measurements of weights and the methods provide an average measurement over a period of 7 - 14 days, which reduces the imprecision caused by day-to-day variability. Taken together, it is plausible that previously published estimated of milk intake in exclusively breast-fed infants based largely on test-weighing studies might have been too low due to bias in the test-weighing method.

The present study found no differences in milk intake between exclusively breast-fed and complementary breast-fed infants at the 2nd time-point. In fact, four of the six complementary breast-fed infants could be categorised as predominantly breast-fed. Haisma and colleagues also found no difference in milk intake between exclusively and predominantly breast-fed infants (n = 51) in a study using deuterium dose-to-mother²⁸.

5.4.3 Differences in milk intake by study design

Overall, we found that milk intake increased over time (significantly for boys) with a mean increase of 65 g/d between time-points. The systematic review by Reilly and colleagues indicated a difference in daily milk intake between cross-sectional time-points (3 - 4 months and 6 months) at around 115 g/d³. The data compiled by Butte and colleagues also indicated an increase in milk intake over time - but this also included mainly cross-sectional studies⁴².

The review by de Costa and colleagues did not find a significant increase in milk intake over the ages 3 - 6 months from the deuterium dose-to-mother studies, but they did not present results of longitudinal and cross-sectional studies separately. We have not been able to find any other study that has measured milk intake longitudinally from the developed world using an isotopic method in infants that were exclusively breast-fed to 6 months of age, as defined by WHO². These data only seem to be available as cross-sectional measurements using test-weighing, or including predominantly breast-fed infants.

It is a commonly accepted notion, that breast milk output plateaus after about 3 - 4 months of age³. The nine longitudinal test-weighing studies included in the systematic review by Reilly and colleagues³ all reported no marked increase in milk intake over time. Hence, the observations of higher milk intake over time found in the cross-sectional studies included in the systematic review have not previously been confirmed in longitudinal studies, except in the DONALD study, where they found a significant increase in milk intake between 3 and 6 months of age in exclusively breast-fed girls, measured by test-weighing²⁸¹.

Cross-sectional studies may incur a risk of selection bias because infant weight is inversely associated with duration of breastfeeding^{425,426}. Therefore, larger infants are more likely to be introduced to complementary foods before 6 months, and any sample of infants selected at 6 months for still being exclusively breast-fed, is more likely to consist of smaller infants with lower energy requirements and milk intakes. Therefore, cross-sectional studies at 6 months might be selection biased in a way that affects the measurement of milk intake and provide a lower estimate of milk intakes at 6 months. A longitudinal study eliminates such selection bias. The participants in the present study were chosen for their determination to persevere with exclusive breast-feeding until 6 months. This participant selection is most likely behavioural and would therefore only bias the results of milk intake through breast-feeding behaviour (Chapter 7).

5.4.4 Milk energy content

The (metabolisable) milk energy content in the present study was not significantly different from the value found in the systematic review by Reilly and colleagues (2.6 kJ/g), which was derived from pooling the results from 25 studies across the ages of 3 - 6 months³. The majority of these studies (22 studies) used milk sampling and measured (bomb calorimetry) or estimated (published values of gross energy content of chemical constituents) gross milk energy content, which was then corrected for metabolisability by a factor 0.93³³⁶. Milk sampling regimes varied greatly across studies, and it was not possible to carry out a formal analysis of the variation in milk energy content according to sampling regime³.

Due to the high variability in breast milk composition^{290,291,427}, any sampling regime incurs a risk of obtaining an unrepresentative sample of breast milk, whereas isotopic methods provide an average. However, after correcting for metabolisability, Reilly and colleagues did not find any difference in milk energy content between the three studies using an isotopic method and the 22 studies using milk sampling³.

The present study did not find any change over time in milk energy content which could contribute to an increasing milk energy intake during exclusive breast-feeding. Mandel and colleagues found that milk energy content and crematocrit levels were higher in milk from mothers that had breast-fed for a prolonged duration (>12 months) compared to mothers that had breastfed for 2 - 6 months²⁹⁴, but this comparison was based on cross-sectional data. A longitudinal study found increases in energy and lipid content of breast milk increased between 2 and 16 weeks of lactation⁴²⁸. However, another longitudinal study published by Mitoulas and colleagues found decreasing milk fat and energy content between 1 and 4 months, no significant change between 4 and 6 months, and then increasing fat and energy content by 9 and 12 months^{285,429}. These findings were all based on milk sampling, and are thus prone to imprecision caused by the variability in breast milk composition²⁷⁷. In contrast, the authors of the Davis Area Research on Lactation, Infant Nutrition and Growth (DARLING) study did not find any significant changes in milk fat and energy content between 3 and 12 months, where they used complete evacuation of the breast to obtain milk samples¹²⁵. Taking short term biological variations into account, it is probable that milk energy content remains fairly stable over time.

5.4.5 Metabolisability of breast milk

There is a possibility of an increase in metabolisability, i.e. the fraction of the milk energy content that is available for energy metabolism. In early infancy, the gut goes through a major maturation and development, and the effect of feeding on this has been studied using piglets as animal models¹⁶⁰. It is possible, that infant gut maturation could result in increasing metabolisability of breast milk. A population study finding decreasing stool frequency⁴³⁰ (and therefore decreasing faecal energy loss) with infant age, and a review noting that faecal fat excretion declines during the first few months of infancy²⁵², would support this notion.

Furthermore, pooled data of specimens obtained from diseased infants have revealed evidence of an 'immature' pancreas such that lipase levels do not seem to reach normal adult levels until a few months after birth⁴³¹. If this is true, it could affect lipid absorption and hence metabolisability of breast milk.

Metabolisability has only been investigated in one study by Southgate and Barrett in 1966³³⁶, who investigated ten newborn breast-fed infants (11 to 17 days old) for gross milk intake, gross milk energy content and gross energy output from complete stool collection, and calculated metabolisability as energy intake divided by energy output (kJ/d). They found a mean \pm SD metabolisability of $91.8 \pm 2.7\%$ in breast-fed infants³³⁶. If metabolisability increases with infant age, this will be reflected within the measured metabolisable milk intake, but not in any gross measurements of milk intake by the test-weighing method.

As the present study only measured metabolisable milk intake, the observed change in milk intake could be due to either a change in gross volume of breast milk or an increase in metabolisability, or both. Hence, an increasing metabolisability could help explain why longitudinal test-weighing studies fail to find an increase in milk intake over time, when they use a constant factor to account for metabolisability³ or ignore the issue of metabolisability altogether. If metabolisability is in fact increasing with age, the gross volume of milk intake may appear to be levelling out, while the infant is in fact getting an increasing amount of energy from this milk. Given this important methodological difference in measuring gross and metabolisable milk intake, it would be interesting to explore whether metabolisability changes with infant age by replicating the study by Southgate and Barrett³³⁶ in an older population of exclusively breast-fed infants, and preferably as a longitudinal study.

5.4.6 Milk intake relative to infant sex, age and size

The differences in milk intake between sexes found in the present study were noticeable, but it is not a new finding. Both the study by Da Costa and colleagues and the review by Butte and colleagues found significantly higher milk intake for boys than girls^{42,270}. In the present study, boys increased significantly in milk intake between time-points, while the change was not statistically significant in girls.

However, the First-Feed study was not designed to be powered to reveal differences in variables of milk intake between sexes - hence this analysis was only exploratory. A larger sample size may be all that is needed to detect a significant increase in milk intake between time-point for girls also. In contrast to the present study, the DONALD study found an increase in milk intake between 3 and 6 months in exclusively breast-fed girls, but not in boys, measured by 3-day test-weighing²⁸¹.

To maximise power in the present study, the variables were stacked when exploring relationships between milk intake and infant age and size³⁶². Infant age only had a weak positive correlation with milk intake in g/d. Da Costa and colleagues found strong correlations between milk intake and infant age, but they grouped their data into age-categories²⁷⁰. In the present study, milk intake (g/d) showed the strongest significant correlations to weight and knee-heel-length and crown-heel-length, which were all positive, while correlations to daily milk intake per kilogram body weight were generally negative. One of the consequences of imprecision in measurements is a reduced strength of correlations to related variables due to the high variability.

5.4.7 Limitations and strengths of the present study

The DLW method has been validated in infants for measurements of TEE in hospital settings with precision of about 5%, and produce very similar results for milk intake when compared to direct measurements of bottle weights in formula-fed infants (section 2.3.1). The imprecision of the DLW method includes analytical error, biological variation, and errors caused by using mean values of correction factors in the calculation of outcome variables³²⁵. For instance, we used a standard RQ for a Western diet³³⁴ rather than the RQ obtained specifically for breast milk²⁴², in order to improve comparability with other studies (Appendix B.5). Finally, the precision of the DLW method was further reduced in the present study by dose spills (Chapter 4) - particularly compared to the hospital setting used in the validation studies, where no spills were encountered as they used *in situ* naso-gastric tubes^{246,306}.

In the present study we found the calculations of daily milk intake were normally distributed, but the SD's were wider for girls than boys. This high variability was also present when daily milk intake was expressed per kg body weight.

Therefore, it was not explained by a wide variation in the body weight of the girls in the study, and thus it may just be imprecision in the measurement of milk intake. The high variability in the milk intake of girls reduced the power to test the hypotheses for this population group in the present study, but the study was sufficiently powered to test the primary hypotheses of change in milk intake between the two time-points. There is also the risk of milk intake being over-estimated in the present study due to other non-milk water intake - a risk that could have been avoided using the deuterium dose-to-mother method for measuring milk intake.

However, any overestimation of milk intake due to intake that was not breast milk would only influence the measurement of breast milk intake, but not the measurement of TEE. Therefore, milk energy content, which is calculated from milk intake and TEI (TEE plus E_{growth}) would be under-estimated, and the present study found very similar values for milk energy content as the value obtained in the systematic review by Reilly and colleagues³. Although this does not exclude the possibility that some mothers may not have complied fully with the WHO definition of exclusive breast-feeding, it does indicate that this was not a prevalent problem in the present sample of participants.

The major strengths of the present study were the longitudinal design lasting to 6 months of age as well as the opportunity to use a state-of-the-art isotopic technique, instead of test-weighing, to measure variables of milk intake, and to simultaneously obtain variables of infant energy balance. These variables of infant energy balance in infants exclusively breast-fed will be explored further in the next chapter.

5.5 Summary

The present study was the first to use a longitudinal design combined with an objective method to measure milk intake in infants successfully exclusively breast-fed to 6 months, which is an important addition to the existing literature both in terms of design and methodology. It suggests that milk intake during exclusive breast-feeding to 6 months is higher than previously thought and increases over time. Therefore, the possible explanation for the shortfall in energy supply during exclusive breast-feeding at 6 months as identified in the Reilly-Wells hypothesis⁴ is supported by this study.

CHAPTER 6 INFANT ENERGY BALANCE

6.1 Energy balance and energy requirements

The present chapter reports on energy balance of the infants in the First-Feed study. The variables of energy balance included TEE, E_{growth} and body composition (and rates of change in body composition) variables which were described as well as TEI which was compared to reference values of infant energy requirements at the group level. Furthermore, equations to predict TEE and infant energy requirements were tested against the present data for their accuracy at the group level and explored for errors at the individual level. Some of these results generated a paper on validation of energy requirement equations for exclusively breast-fed infants¹⁷.

6.1.1 The Reilly-Wells hypothesis on energy supply

As described in the introduction (section 1.1.2), exclusive breast-feeding was deemed adequate by Butte and colleagues⁴² to fulfil infant energy requirements for the first 6 months of life, although empirical evidence is limited on energy requirements of exclusively breast-fed infants beyond 3 months of age^{42,432}. Subsequently, Reilly and Wells combined the data on mean milk intake at 6 months of age and mean milk energy content from exclusively breast-fed infants from the systematic review³ to produce a mean energy intake of 2.2 to 2.4 MJ/d⁴ at 6 months during exclusive breast-feeding. In comparison, the FAO/WHO/UNU references for mean infant energy requirements are 2.5 to 2.7 MJ/d for a 6-month-old infant of typical weight⁷ (50th percentile of the UK 1990 reference²⁹⁶). Chapter 3 revealed that this shortfall in energy supply during exclusive breast-feeding as identified by Reilly and Wells⁴ could not be explained by exclusively breast-fed infants being unusually small and/or growing slowly, but Chapter 5 has suggested that metabolisable milk intakes higher than literature values and increasing over time may offer an explanation to the Reilly and Wells hypothesis³. However, determining what infant energy requirements are during normal growth in exclusively breast-fed infants might also help to explain how energy metabolism is balanced in this population group.

6.1.2 Reference values for total energy expenditure and energy requirements

Infant energy requirements

As described in section 1.2.1, infant energy requirements are based on energy needs for basal metabolism, thermogenesis, physical activity energy expenditure, and energy required for growth, and is largely influenced by age, gender and body size²⁵². While TEE includes the energetic costs of synthesising new tissues, the energy that is contained in those new tissues, E_{growth} , needs to be estimated. During infancy, the growth velocity decreases, and the composition of the newly synthesised tissues change¹⁴⁵. Therefore, there are longitudinal changes both in the value (size) and in the components that make E_{growth} , which varies with sex and age^{249,337}, and which influence infant energy requirements. The factorial approach of TEE plus E_{growth} is considered the criterion method for determining infant energy requirements, and this is also the most objective way of measuring TEI during normal growth⁷. This criterion method will be used in the present study to determine infant energy requirements of the infants in the First-Feed study.

References for energy requirements for groups

Human energy requirements published by FAO/WHO/UNU⁷ are intended to be a prescriptive reference at the group level to support and maintain health and good nutritional status. Mean values were published in monthly intervals, for both sexes and for breast-fed and formula-fed infants, and they are frequently cited for reference worldwide⁷ (Table 6.1). However, the values for breast-fed infants were based on data from infants who were not exclusively breast-fed as currently defined and recommended by WHO^{1,2}. Instead, the studies included predominant breast-feeding practised until around 4 months of age. Feeding mode affects TEE, body composition and growth^{140-146,242,260,364,433,434}, and this is likely to influence energy requirements. The evidence was recently updated in the UK by the Scientific Advisory Committee on Nutrition (SACN), with no new evidence on TEE, but incorporating the WHO Child Growth Standards (Table 6.1)⁴³². Therefore, it was an objective of the First-Feed study to determine infant energy requirements in infants that were exclusively breast-fed as defined and recommended by WHO^{1,2}, using the factorial approach.

Table 6.1 Energy requirements (kJ/kg body weight*day) of breast-fed and formula-fed infants as published by FAO/WHO/UNU⁷ and SACN⁴³².

Age	FAO/WHO/UNU				SACN			
	Breast-fed		Formula-fed		Breast-fed		Formula-fed	
	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls
1 month	445	415	510	490	454	434	527	514
2 months	410	395	460	455	435	422	486	480
3 months	380	375	420	420	393	384	432	430
4 months	330	335	360	370	332	336	363	373
5 months	330	330	355	365	329	332	356	364
6 months	325	330	350	355	328	329	351	357

Predictions of TEE and energy requirements for individuals

In many clinical situations, as well as for surveillance of the healthy infant, it can be useful to predict TEE or energy requirements at an individual level. In deriving equations for predicting TEE, FAO/WHO/UNU pooled data from 13 studies, took into account four studies showing TEE to be associated with feeding mode, and generated separate TEE prediction equations for formula-fed and breast-fed infants⁷. The equations for predicting TEE were based on body weight only, and would therefore be feasible to use in the clinical setting.

Similarly, the FAO/WHO/UNU references⁷ provided references for infant energy requirements per kg body weight (Table 6.1), which could be used in the clinical setting as a simple multiplier of body weight for prediction of energy requirements of individual infants - even though they are intended as mean references for comparison at the group level. Similar estimated average requirements were published in 1991 for the UK by the Department of Health⁴³⁵.

Other publications also provide regression equations, which could be used to predict energy requirements at the individual level^{252,260}. While these prediction equations were based on the highest possible quality data (the 4-compartment model), the data set was relatively small (n = 76) and the infants were not exclusively breast-fed according to the current WHO recommendation¹. Hence, there was some doubt as to their applicability in exclusively breast-fed infants.

Given all the promotional efforts to increase breast-feeding exclusivity and duration, it would seem important to assess the accuracy of these methods for predicting TEE and energy requirements in individual infants who are exclusively breast-fed as defined and recommended by WHO^{1,2}.

6.1.3 Aims of the present chapter

The aims of the present chapter were to:

- Measure TEE, estimate E_{growth} , and determine energy requirements using the factorial approach and present growth in body composition in a sample of infants who were exclusively breast-fed as defined by WHO².
- Compare determined energy requirements with references for mean energy requirements published by FAO/WHO/UNU⁷ at the group level.
- Assess the accuracy of predicting TEE at the individual level from body weight using the equation for breast-fed infants⁷ by comparison with measured TEE.
- Assess the accuracy of predicting energy requirements at the individual level, using mean reference energy requirements per kg body weight^{7,435} and regression equations²⁵² by comparison with determined energy requirements using the factorial approach.

6.2 Methods

6.2.1 Measured and predicted total energy expenditure

TEE was measured using the DLW method as described in section 2.3, which is the criterion method for measuring TEE. TEE was also predicted using the equation from the FAO/WHO/UNU publication on TEE for breast-fed infants⁷:

$$\text{TEE}_{\text{pred}} (\text{kJ/d}) = -635 + 388 \times \text{body weight (kg)} \quad \text{SEE} = 453 \text{ kJ/d}$$

6.2.2 Determined and predicted energy requirements

Growth is a sensitive indicator of whether energy requirements are met, and the results from Chapter 3 suggested that the infants in the present sample overall were growing normally relative to the WHO Child Growth Standards. Therefore, determined energy requirements in the present sample were assumed to be equal to metabolisable TEI (hence the terms TEI and determined energy requirements are used interchangeably), and were determined using the factorial approach of adding measured TEE to estimated E_{growth} as described in section 2.3.6.

Individual energy requirements (E_{req1}) were predicted from mean daily energy requirements per kg body weight for breast-fed infants published by FAO/WHO/UNU⁷, using the values of 355 kJ/kg*d (mean of values for 3 and 4 months) at the 1st time-point, and 330 kJ/kg*d (6 months) at the 2nd time-point.

Similarly, individual energy requirements (E_{req2}) were predicted from estimated average requirements per kg body weight (for all infants regardless of feeding mode) published by the UK Department of Health, where the reference values are 420 kJ/kg*d (3 months) and 400 kJ/kg*d (6 months) at the 1st and 2nd time-points, respectively⁴³⁵.

Finally, individual energy requirements were also predicted using empirically derived regression equations²⁶⁰, based on age, sex and feeding mode as well as either body weight (E_{req3}) or body composition; FFM and FM (E_{req4}). Since feeding mode was defined as breast-fed for all infants in the present study, the equations were revised to include the factor for feeding mode in the constant.

Prediction of energy requirements from age, sex, and body weight were:

$$E_{\text{req3}} \text{ (kJ/d)} = 460 + 13\text{age} - 47\text{sex} + 277\text{weight}$$

Prediction of energy requirements from age, sex, and body composition were:

$$E_{\text{req4}} \text{ (kJ/d)} = 383 + 2\text{age} - 7\text{sex} + 358\text{FFM} + 118\text{FM},$$

where sex was denoted 1 for boys and 2 for girls, age was in months (calculated with one decimal place as age in days divided by 30.4 days/month), and body weight, FFM and FM was in kg.

6.2.3 Statistical methods

Power considerations

The sample size for the First-Feed study was based on the power to detect a longitudinal difference in breast milk intake (section 2.7.1). For comparison, a review by Butte²⁵² including DLW studies from the first year of infancy indicated the typical sample size to be around 40 infants in each study. For the study of normative body composition data during infancy, Butte and colleagues included 76 infants (40 breast-fed) studied longitudinally¹⁴⁵.

Due to other studies finding differences between sexes, post hoc sample size calculations were done to get an indication of whether the observed difference was likely to have been significant, if the sample had been larger. If the required sample size to detect a difference between sexes was found to be very large, it can be taken as an indication that the difference is not real and power is very low.

Statistical analyses

The outcome variables for the present chapter were measured TEE, estimated E_{growth} , determined energy requirements as well as body composition and growth in body composition variables, which were described using summary statistics (section 2.7.2). Data-driven tests for differences between sexes (e.g. expecting higher TEI for boys than girls) were examined using independent t-tests, and longitudinal changes in outcome variables were tested using paired t-tests. Outcome variables were also tested for differences from mean reference values^{7,435} at the group level using 1-sample t-tests, where appropriate.

Further outcome variables were predicted TEE (TEE_{pred}) and predicted energy requirements ($E_{\text{req1}} - E_{\text{req4}}$) at the individual level, which were compared to measured TEE and determined energy requirements, respectively, using paired t-tests (mean difference and 95% CI). In addition, the accuracy of the prediction equations was assessed using Pearson correlations as well as Bland-Altman plots³²⁴ with calculation of bias and limits of agreement defined as the mean difference $\pm 2SD$.

6.3 Results

6.3.1 Descriptive variables of energy balance

Variables of body composition, growth, TEE and TEI are summarised in Table 6.2. In general, variables were normally distributed, with exception of a few variables for boys due to one extreme outlier. As there was no fault identified for this outlier, it was kept in the dataset. This resulted in more variation in some variables for boys compared to girls.

Table 6.2 Energy balance variables (mean \pm SD) at the 1st and the 2nd time-point.

	1 st time point			2 nd time point		
	Boys n = 17	Girls n = 19	All n = 36	Boys n = 18	Girls n = 20	All n = 38
Body Composition						
FFM, g	4961 \pm 561	4676 \pm 470	4811 \pm 527	5680 \pm 589	5215 \pm 551 ^c	5436 \pm 609 ^a
FM, g	1851 \pm 400	1732 \pm 378	1788 \pm 388	2216 \pm 486	2193 \pm 414	2204 \pm 443 ^a
FM%, %	27.1 \pm 4.4	26.9 \pm 4.2	27.1 \pm 4.2	28.0 \pm 4.7	29.5 \pm 3.9	28.8 \pm 4.3 ^b
Weight gain, g/d	25.6 \pm 13.6	22.9 \pm 12.0	24.2 \pm 12.6	21.5 \pm 14.7	15.9 \pm 12.0	18.6 \pm 13.4
FFM gain, g/d	12.6 \pm 9.6	9.6 \pm 8.1	11.0 \pm 8.8	15.7 \pm 9.7	9.2 \pm 8.4 ^c	12.3 \pm 9.5
Protein gain, g/d	2.2 \pm 1.5	1.8 \pm 1.3	2.0 \pm 1.4	2.7 \pm 1.5	1.8 \pm 1.4	2.2 \pm 1.5
FM gain, g/d	13.1 \pm 4.3	13.3 \pm 4.2	13.2 \pm 4.2	5.8 \pm 5.6	6.7 \pm 3.8	6.3 \pm 4.7 ^a
Protein stored, kJ/d	53 \pm 35	43 \pm 31	48 \pm 33	65 \pm 37	42 \pm 33	53 \pm 36
Fat stored, kJ/d	505 \pm 165	513 \pm 161	509 \pm 161	225 \pm 217	260 \pm 146	244 \pm 181 ^a
E_{growth}, kJ/d	558 \pm 198	556 \pm 189	557 \pm 191	289 \pm 247	303 \pm 176	296 \pm 210 ^a
Energy Expenditure						
TEE, kJ/d	2024 \pm 340	1846 \pm 173	1930 \pm 276	2458 \pm 465	2146 \pm 235 ^c	2294 \pm 391 ^a
TEE, kJ/kg*d	303 \pm 47	294 \pm 39	299 \pm 42	316 \pm 64	294 \pm 35	304 \pm 52
TEE/TEI, %	78.4 \pm 7.3	77.1 \pm 6.8	77.7 \pm 6.9	89.7 \pm 8.0	88.0 \pm 6.0	88.8 \pm 7.0 ^a
E_{growth}/TEI, %	21.6 \pm 7.3	22.9 \pm 6.8	22.3 \pm 6.9	10.3 \pm 8.0	12.0 \pm 6.0	11.2 \pm 7.0 ^a
Energy requirements						
TEI, kJ/d	2582 \pm 362	2403 \pm 215	2487 \pm 303	2748 \pm 480	2449 \pm 312 ^c	2590 \pm 243
TEI, kJ/kg*d	380 \pm 40	377 \pm 29	378 \pm 34	350 \pm 65	332 \pm 40	340 \pm 53 ^b

Differences between time-points (paired t-tests, n = 33; ^a P <0.001, ^b P <0.01.

Differences between sexes (independent t-tests, n = 38; ^c P <0.05

FFM: Fat free mass, FM: Fat mass, TEE: Total energy expenditure, TEI: Total energy intake.

Differences in energy balance between sexes

The present study was fortunate to have a fairly equal distribution of boys and girls, and Levene's tests showed homogeneity of variance for almost all variables. However, the sample size was too small to detect a statistically significant difference between sexes for most of the variables, as the study was not specifically powered to explore this issue. In general, differences between sexes at the 1st time-point were only trends, but most of these trends were also present at the 2nd time-point, where some had reached statistical significance.

A series of post hoc sample size calculations were performed with a fixed power of 0.8 and a two-sided significance level of <0.05, and Cohen's delta was the detected mean difference between sexes divided by the SD of the boys (as this was the greatest) for each variable, which were entered into an internet based sample size calculator³⁶⁷. The sample sizes ranged from 37 infants per group as the lowest (to detect the mean difference in protein gain at the 2nd time-point between groups as significant) and up to around 157,000 infants per group as the highest (to detect the mean difference in E_{growth} at the 1st time-point as significant). The majority of the post hoc power calculations suggested sample sizes of between 100 and 500 infants in each group to detect between-sex differences due to the high variability in the data, i.e. the power of the present study to explore differences between sexes was very low.

Boys had significantly higher TEE and TEI at the 2nd time-point. They tended to have higher FM and FFM, and this was statistically significant for FFM at the 2nd time-point (5.7 kg versus 5.2 kg, $P < 0.05$). Furthermore, boys also had a significantly higher rate of FFM gain (15.7 g/d versus 9.2 g/d, $P < 0.05$) than girls at the 2nd time-point. There was a trend towards higher rate of weight gain and protein gain for boys at both time-points, while rate of FM gain was very similar at the 1st time-point and then tended to be higher for girls at the 2nd time-point. Hence the girls tended to store slightly more energy than boys at the 2nd time-point, mostly as fat. Generally, the differences between boys and girls were larger at the 2nd time-point than at the 1st time-point.

Changes over time in energy balance

TEE for the whole group of infants was 1930 kJ/d at the 1st time-point and increased to 2294 kJ/d at the 2nd time-point ($P < 0.001$).

TEE per kg body weight remained unchanged over time. TEE as proportion of TEI increased significantly from 77.7% to 88.8% ($P < 0.001$), while the proportion of TEI that was E_{growth} halved from 22.3% to 11.2% ($P \leq 0.001$). There was a trend for rate of weight gain to decrease between time-points, but this was not statistically significant.

As weight increased with age, both FFM and FM increased significantly between time-points ($P < 0.001$ for both). Although FFM increased more than FM in absolute value, relative to body weight FM increased more. This was evident as a significant increase in FM% for the whole sample of infants, from 27% to 28% in boys and from 27% to 29.5% in girls (both $P < 0.01$).

The rate of fat gain and the rate of energy stored as fat more than halved from the 1st to the 2nd time-point. E_{growth} decreased from 557 kJ/d to 296 kJ/d ($P < 0.001$). Rates of FFM gain and protein gain tended to increase.

6.3.2 Determined energy requirements at the group level

From the references published by FAO/WHO/UNU⁷, mean energy requirements for boys should be 2380 kJ/d and 2674 kJ/d at 3 - 4 months and 5 - 6 months respectively. This was significantly lower than the present study with a mean difference (95% CI) of 202 (15 to 388) kJ/d ($P = 0.036$) for the 1st time-point, while there was no significant difference at the 2nd time-point (mean difference (95% CI) 74 (-165 to 312) kJ/d, $P = 0.524$).

For girls the mean energy requirements should be 2245 kJ/d and 2507 kJ/d at 3 - 4 months and 5 - 6 months, respectively. Similarly, this is significantly lower than the present study with a mean difference (95% CI) of 158 (54 to 262) kJ/d ($P = 0.005$) at the 1st time-point, while it is not significantly different at the 2nd time-point (mean difference (95% CI) -58 (-204 to 88) kJ/d, $P = 0.415$).

6.3.3 Prediction of total energy expenditure at the individual level

Variables of predicted TEE and energy requirements (to compare with measured TEE and determined energy requirements) are presented in Table 6.3, and an overview of the statistical analyses can be found in Table 6.4.

Table 6.3 Predicted variables of TEE and infant energy requirements (mean \pm SD).

	1 st time point			2 nd time point		
	Boys n = 17	Girls n = 19	All n = 36	Boys n = 18	Girls n = 20	All n = 38
Energy Expenditure						
Measured TEE, kJ/d^a	2024 \pm 340	1846 \pm 173	1930 \pm 276	2458 \pm 465	2146 \pm 235	2294 \pm 391
Predicted TEE, kJ/d	1968 \pm 294	1818 \pm 257	1889 \pm 282	2402 \pm 297	1213 \pm 306	2303 \pm 312
Energy Requirements						
Determined TEI^a	2582 \pm 362	2403 \pm 215	2487 \pm 303	2748 \pm 480	2449 \pm 312	2590 \pm 243
Predicted E_{req1}^b	2381 \pm 269	2244 \pm 235	2309 \pm 258	2583 \pm 252	2423 \pm 260	2499 \pm 266
Predicted E_{req2}^c	2817 \pm 319	2655 \pm 278	2732 \pm 305	3131 \pm 306	2937 \pm 315	3029 \pm 322
Predicted E_{req3}^d	2317 \pm 210	2164 \pm 184	2236 \pm 209	2655 \pm 210	2474 \pm 217	2560 \pm 230
Predicted E_{req4}^d	2356 \pm 214	2237 \pm 180	2293 \pm 203	2660 \pm 220	2492 \pm 215	2572 \pm 230

Values used for prediction equations were taken as values on day 0 of the DLW measurement.

^a From Table 6.2.

^b Energy requirements determined using the factorial approach and predicted using the mean reference from FAO/WHO/UNU⁷ as a multiplier of body weight.

^c Energy requirements determined using the factorial approach and predicted using the mean reference from UK 1991⁴³⁵ as a multiplier of body weight.

^d Energy requirements determined using the factorial approach and predicted using a regression equation²⁶⁰.

Table 6.4 Relationship and agreement between criterion methods and predictions for TEE and energy requirements.

	Pearson correlation		Paired t-test			Limits of agreement	
	r	P	Mean difference (kJ/d)	SD (kJ/d)	P	Lower (kJ/d)	Upper (kJ/d)
1st time point (n = 36)							
Measured versus predicted TEE ^a	0.39	0.018	-42	308	0.423	-658	574
Determined TEI versus E _{req1} ^b	0.70	<0.001	-178	221	<0.001	-620	264
Determined TEI versus E _{req2} ^c	0.70	<0.001	244	235	<0.001	-226	714
Determined TEI versus E _{req3} ^d	0.71	<0.001	-251	212	<0.001	-675	173
Determined TEI versus E _{req4} ^d	0.69	<0.001	-194	219	<0.001	-631	245
2nd time point (n = 38)							
Measured versus predicted TEE ^a	0.36	0.025	9	406	0.889	-795	813
Determined TEI versus E _{req1} ^b	0.41	0.010	-91	395	0.162	-883	745
Determined TEI versus E _{req2} ^c	0.41	0.010	439	412	<0.001	385	1263
Determined TEI versus E _{req3} ^d	0.43	<0.001	-31	384	0.624	-798	738
Determined TEI versus E _{req4} ^d	0.55	<0.001	-19	354	0.746	-727	689

^a TEE measured by doubly-labelled water and predicted using prediction equation published by FAO/WHO/UNU⁷.

^b Energy requirements determined using the factorial approach and predicted using the mean reference from FAO/WHO/UNU⁷ as a multiplier of body weight.

^c Energy requirements determined using the factorial approach and predicted using the mean reference from UK 1991⁴³⁵ as a multiplier of body weight.

^d Energy requirements determined using the factorial approach and predicted using a regression equation²⁶⁰.

Measured TEE and predicted TEE from the equation by FAO/WHO/UNU⁷ were significantly positively correlated at both time-points (Table 6.4). The mean (95% CI) paired difference between predicted and measured TEE was -42 (-146 to 63) kJ/d at the 1st time-point ($P = 0.423$), and 9 (-123 to 141) kJ/d at the 2nd time-point ($P = 0.889$). Excluding the extreme outlier, the paired mean difference was 50 (-56 to 156) kJ/d, $P = 0.348$, at the 2nd time-point. Bland-Altman plots of the differences between predicted and measured TEE versus mean of the predicted and measured TEE are shown in Figure 6.1 for both time-points. Limits of agreement were -658 to 574 kJ/d at the 1st time-point, and -795 to 813 kJ/d at the 2nd time-point. The errors in the prediction of TEE were significantly negatively correlated with the measured TEE ($r = -0.62$ and $r = -0.76$ at the 1st and 2nd time-points, $P < 0.001$ for both).

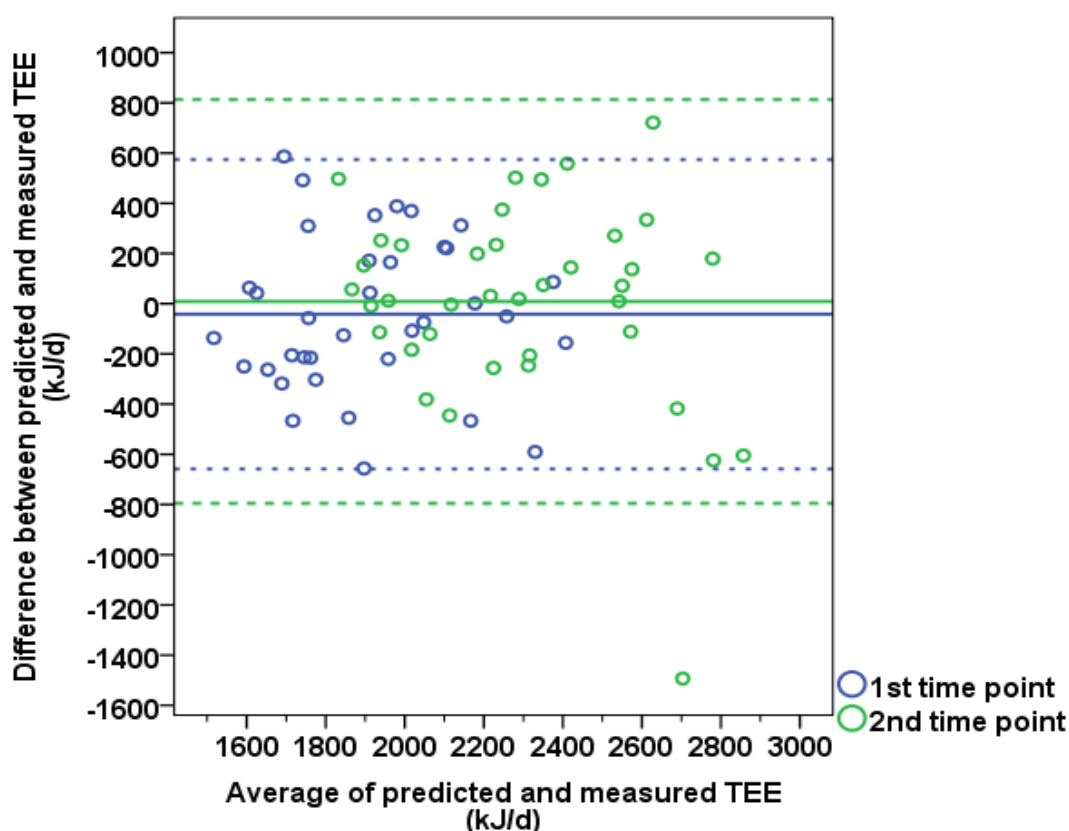


Figure 6.1 Bland-Altman plot of difference between predicted and measured TEE versus the mean of predicted and measured TEE.

Full lines indicate mean difference for both time-points (blue for the 1st time-point and green for the 2nd time-point), while limits of agreement are marked in dotted lines.

TEE: Total energy expenditure.

6.3.4 Prediction of energy requirements at the individual level

Prediction of energy requirements using a multiplier of body weight

Energy requirements predicted using the FAO/WHO/UNU reference⁷ as a multiplier of body weight, E_{req1} , were significantly positively correlated with determined energy requirements at both time-points ($r = 0.70$, $P < 0.001$ and $r = 0.41$, $P = 0.010$, at the 1st and 2nd time-points, respectively). At the 1st time-point, E_{req1} was significantly lower than determined energy requirements. The mean (95% CI) paired difference was -178 (-253 to -104) kJ/d ($P < 0.001$). At the 2nd time-point, the mean (95% CI) paired difference was not significant (-91 (-221 to 39) kJ/d, $P = 0.162$). Excluding the extreme outlier, the mean (95% CI) paired difference was -53 (-160 to 54), $P = 0.322$. Bland-Altman plot (Figure 6.2) shows the limits of agreement as -620 to 264 kJ/d and -883 to 745 kJ/d at the 1st and 2nd time-points, respectively.

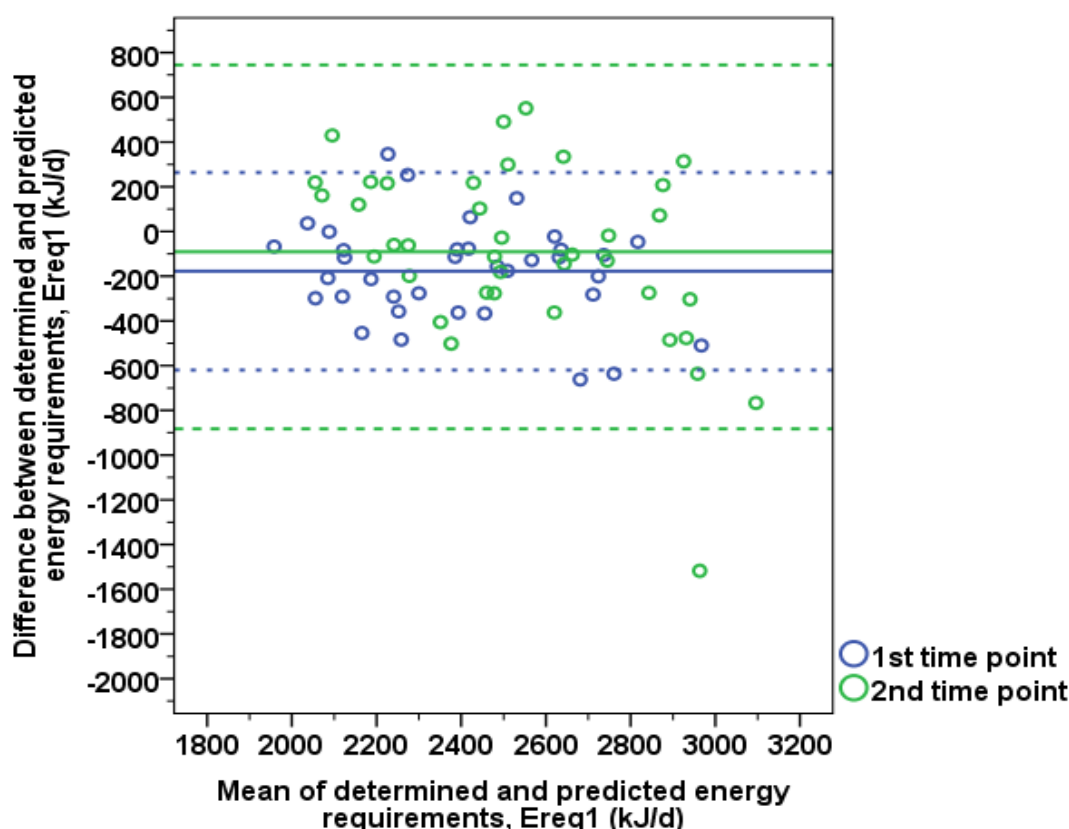


Figure 6.2 Bland-Altman plot of difference between predicted energy requirement (E_{req1}) from the FAO/WHO/UNU reference⁷ and determined energy requirements (TEI) versus the mean of predicted and determined energy requirements.

Full lines indicate mean difference for both time-points (blue is 1st time-point, green is 2nd time-point), while limits of agreement are marked in dotted lines.

E_{req1} : Predicted energy requirements from reference by FAO/WHO/UNU⁷.

Energy requirements predicted using the UK 1991 reference⁴³⁵ as multiplier of body weight, E_{req2} , were also significantly positively correlated with TEI at both time-points with identical correlation coefficients as above (both predictions were based on body weight only, see Table 6.4). E_{req2} was significantly higher than determined energy requirements at both time-points, as the mean (95% CI) paired differences were 244 (165 to 324) kJ/d and 439 (303 to 574) kJ/d (both $P < 0.001$). Excluding the extreme outlier, the mean (95% CI) paired difference was 479 (368 to 590) kJ/d, $P < 0.001$. Bland-Altman plot is shown in Figure 6.3. The limits of agreement were -226 to 714 at the 1st time-point, and -385 to 1263 kJ/d at the 2nd time-point.

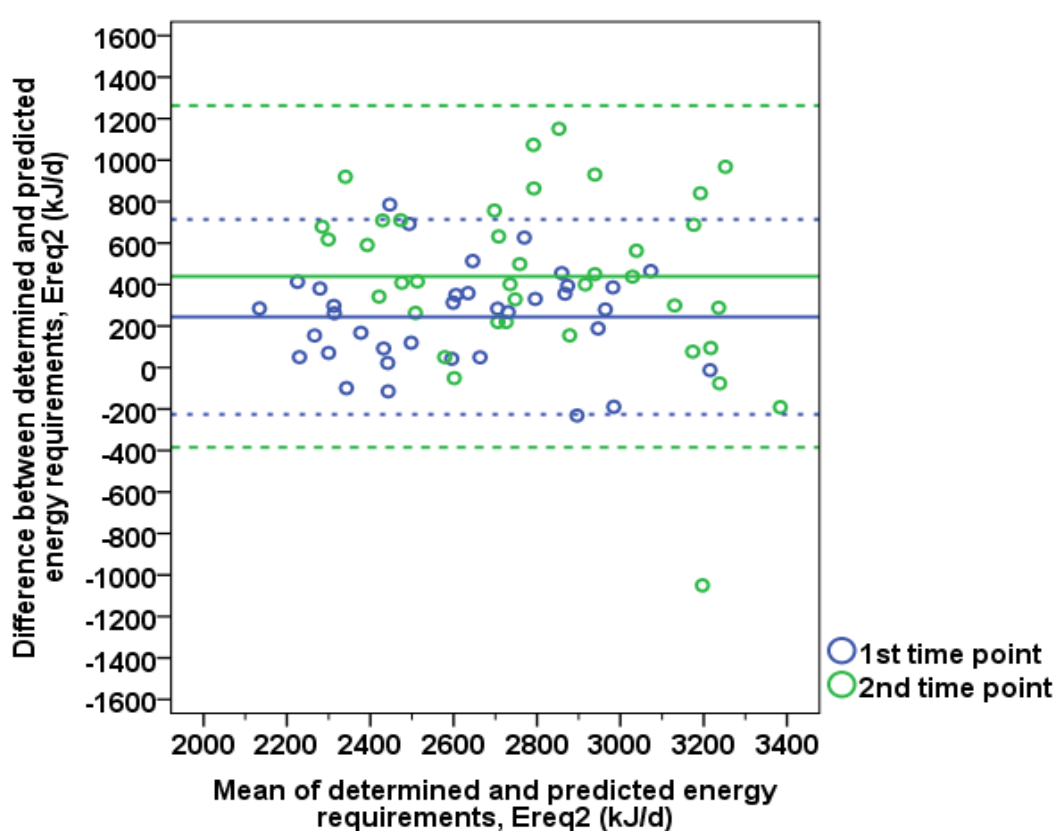


Figure 6.3 Bland-Altman plot of difference between predicted energy requirement (E_{req2}) from the UK 1991 reference⁴³⁵ and determined energy requirements (TEI) versus the mean of predicted and determined energy requirements.

Full lines indicate mean difference for both time-points (blue is 1st time-point, green is 2nd time-point), while limits of agreement are marked in dotted lines.

E_{req2} : Predicted energy requirements from reference from the UK 1991 reference⁴³⁵.

The errors in predicting energy requirements from a multiplier of body weight were significantly negatively correlated with determined energy requirements for both prediction equations (1st and 2nd time-point: E_{req1} ; $r = -0.56$ and $r = -0.79$ both $P < 0.001$, E_{req2} ; $r = -0.38$, $P = 0.220$ and $r = -0.70$, $P < 0.001$).

Prediction of energy requirements using regression equations

Similarly, the predicted energy requirements from regression equations including age, sex and either body weight (E_{req3}) or body composition (E_{req4})²⁶⁰ also showed significant positive correlations with determined energy requirements at both time-points (Table 6.4). At the 1st time-point, both regression equations produced predicted energy requirements that were significantly lower than determined energy requirements, while at the 2nd time-point the mean differences between predicted and determined energy requirements were not significant (Table 6.4).

Bland-Altman plots for the regression equations using age, sex and body weight (E_{req3}) or body composition (E_{req4}) are shown in Figure 6.4 and Figure 6.5, respectively. The limits of agreement for E_{req3} were -675 to 173 kJ/d and -798 to 738 kJ/d at the 1st and 2nd time-points, respectively.

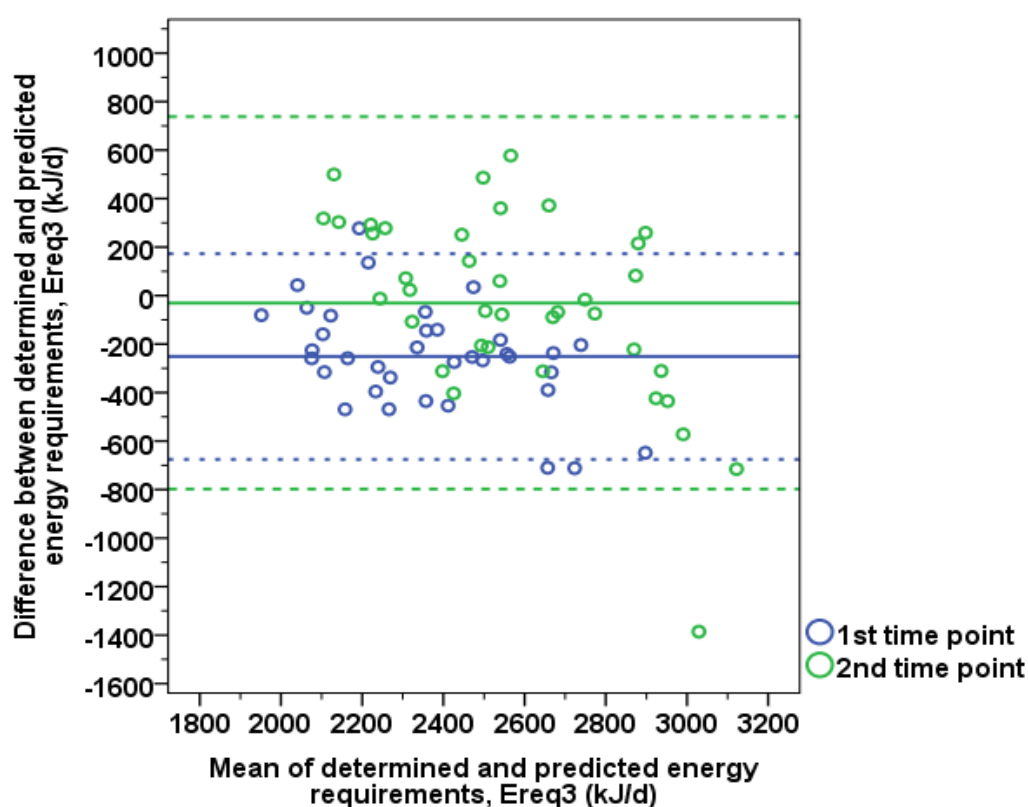


Figure 6.4 Bland-Altman plot of difference between predicted energy requirement (E_{req3}) from sex, age and body weight²⁶⁰ and determined energy requirements (TEI) versus the mean of predicted and determined energy requirements.

Full lines indicate mean difference for the two time-points, while limits of agreement are marked in dotted lines. E_{req3} : Predicted energy requirements from sex, age and body weight.

The limits of agreement for E_{req4} were -631 to 245 kJ/d and -727 to 689 kJ/d at the 1st and 2nd time-points, respectively. The errors were negatively correlated with determined energy requirements (1st and 2nd time-point, respectively: E_{req3} ; $r = -0.73$ and $r = -0.84$, E_{req4} ; $r = -0.74$ and $r = -0.84$, all $P < 0.001$).

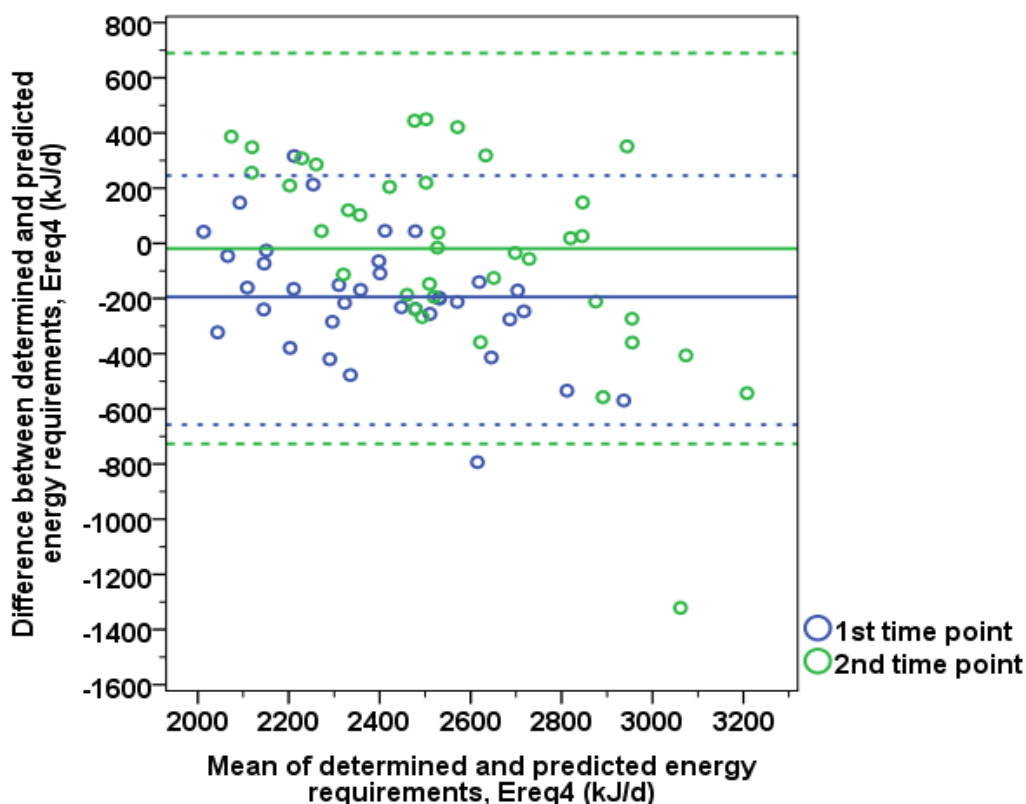


Figure 6.5 Bland-Altman plot of difference between predicted energy requirement (E_{req4}) from sex, age and body composition²⁶⁰ and determined energy requirements (TEI) versus the mean of predicted and determined energy requirements.

Full lines indicate mean difference for both time-points, while limits of agreement are marked in dotted lines. E_{req4} : Predicted energy requirements from sex, age and body composition.

6.4 Discussion

6.4.1 Main findings

In the present chapter, TEE was measured, E_{growth} estimated and energy requirements were determined (as TEI) using the criterion method of the factorial approach. Growth and its components were presented in Table 6.2. At the group level, there was no significant difference between determined energy requirements and mean reference energy requirements by FAO/WHO/UNU⁷ at 6 months of age. This indicates that exclusive breast-feeding to around 6 months of age provides as much energy as recommended according to these references.

The shortfall in energy supply identified in the Reilly-Wells hypothesis comparing mean references for energy requirements based on typical body weight at 6 months of age with mean TEI obtained from literature values of milk intake and milk energy content could therefore not be replicated in the present study, where TEI was determined using the factorial approach. However, at 3½ months of age, the mean reference energy requirements by FAO/WHO/UNU⁷ significantly underestimated energy requirements by around 8% at the group level compared to when energy requirements were determined using the factorial approach.

The prediction equations showed no bias in predicting TEE at either 3½ or at 6 months using the prediction equation published by FAO/WHO/UNU⁷, but individual errors were large - manifested as large limits of agreement - and correlated negatively with the value of TEE at both time-points. The UK 1991 reference⁴³⁵ overestimated energy requirements on average by about 8% and 11% at the 3½ and 6 months of age, respectively. Finally, energy requirement prediction equations based on sex, age and either body weight or body composition performed similar to the FAO/WHO/UNU reference in that they underestimated energy requirements at 3½ months, while there was no bias at 6 months. All prediction equations for energy requirements had errors too great to be used with confidence at an individual level for exclusively breast-fed infants.

6.4.2 References for infant energy requirements at the group level

Published references for energy requirements are intended to be a prescriptive reference at the group level to support and maintain health and good nutritional status, whether published by FAO/WHO/UNU⁷, the UK Department of Health⁴³⁵, or more recently by SACN⁴³². Compared to the reference mean energy requirements published by FAO/WHO/UNU⁷, the group of infants in the present study apparently had an average overconsumption of around 200 kJ/d at 3½ months of age, which is clinically important. At 6 months of age, TEI was not significantly different from reference values, indicating that the infants had an appropriate intake at the group level. However, assessing adequacy of energy intake at group level will not indicate how many infants, if any, or indeed which infants are not getting enough or are getting too much energy on a daily basis.

6.4.3 Performance of prediction equations at the individual level

Prediction of TEE

Predicting TEE at the individual level is notoriously difficult due to the many factors that influence TEE, which are not taken into account in prediction equations, and which increase errors at the individual level. This issue is reflected in the Standard Error of the Estimate of 453 kJ/d, which is stated along with the prediction equation published by FAO/WHO/UNU⁷. Previous studies have shown similar problems in predicting TEE in other populations⁴³⁶⁻⁴³⁸. The FAO/WHO/UNU prediction equation was based on body weight alone, as it was deemed the best predicting factor among other co-varying factors, like length and age⁷. However, other studies have found body size alone to be a poor predictor of TEE during infancy⁴³⁹. Moreover, one study found that FFM and behavioural factors together accounted for 46% of the variation in TEE in 9 and 12 months-olds³⁵³. One study has demonstrated the error of prediction equations for resting metabolic rate can be improved by computing an average from several independently derived prediction equations⁴⁴⁰. This ‘wisdom of crowds’ approach confirmed the weakness of using a simple equation⁴⁴⁰, but the approach needs more equations generated before it can be implemented in clinical practice.

Prediction of energy requirements

The references for infant energy requirements published by FAO/WHO/UNU⁷ are meant to be used at the group level, but are commonly used for predicting energy requirements at the individual level in clinical practice. We found that using this reference as a simple multiplier of body weight, significantly underestimated energy requirements for exclusively breast-fed infants at 3½ months of age, whereas there was no bias at 6 months of age. As there was no bias in predicting TEE at either time-point, it is possible that the equations tested in the present study for predicting energy requirements in infancy do not sufficiently take into account the different growth patterns of exclusively breast-fed infants^{140,141,144,242}. Growth rate is slower at 6 months than at 3 months of age, and constitutes a smaller part of infant energy requirements. In the present study, $E_{\text{growth}}/\text{TEI}$ halved between the two time-points.

The reference published by the UK Department of Health in 1991⁴³⁵ predicted energy requirements to be on average around 8% and 11% higher at 3½ and 6 months, respectively, than the energy requirements determined in the present study by the factorial approach. The UK 1991 reference was partly based on studies of energy intake in formula-fed infants, and it did not take into account the different growth patterns between formula-fed and breast-fed infants⁴³⁵. If these predicted energy requirements were translated into energy intake for a typical infant, this would result in an overconsumption, which may be clinically important. More recently, the SACN published an updated reference on human energy requirements⁴³². For infants, the mean reference energy requirements were reviewed to be based on the evidence from the FAO/WHO/UNU reference⁷, as well as taking into account the more recent evidence of normal growth as revealed in the WHO Child Growth Standards³⁶⁹, but no further evidence has been generated on energy requirements using the factorial approach in infants breast-fed as currently defined² and recommended¹ by WHO.

Butte and colleagues²⁶⁰ performed a thorough study (using the 4-compartment model) and were able to generate regression equations for infant energy requirements that took feeding mode into account as a factor in determining energy requirements, although the breast-fed infants in this study were not breast-fed as currently recommended by WHO. These regression equations resulted in the lowest individual errors of all prediction equations, tested in the present study, when they were compared to energy requirements determined using the factorial approach, but the limits of agreement were still wide.

For all prediction equations tested in the present study, errors at the individual level were large. It has been noted before by Weekes⁴⁴¹, that even when prediction equations are accurate in predicting the average energy requirement at the group level, the individual predictive value is often poor⁴⁴². Therefore, when used in the clinical setting, lack of consideration of such large errors might result in over- or underestimation of energy requirements (section 8.3.1). Furthermore, in the present study, errors were negatively correlated with energy requirements, suggesting that the underestimation was greater when energy requirements were high. This is important to bear in mind whenever prediction equations are used at the individual level, e.g. in the clinical setting.

It is noteworthy that the DONALD study in predominantly breast-fed infants found energy intakes from milk intake measured by 3d-test-weighing very similar to the FAO/WHO/UNU reference for infant energy requirements at 3 months of age, when predicted using same approach as in the present study²⁸¹. However, it is possible that the energy intake was under-estimated using the test-weighing method.

6.4.4 Limitations and strengths of the present study

The present study had a number of limitations. The precision of the estimate of E_{growth} relies on repeated measurements of weight to determine weekly weight gain, and we observed a relatively wide variation in the data on weight gain. This could partly be due to lack of precision of the scale for measuring weight; however, more precise weighing scales may not be suitable for use under field conditions, as it may affect their calibration. More importantly, the daily fluctuations in body weight are much greater than the weekly weight gain for each infant, particularly at 6 months, and, as it was not possible to standardise the weighing relative to e.g. feeding times, this might have introduced an extra large factor of imprecision in the estimate of E_{growth} .

We also observed a larger variation in data of measured TEE as well as the estimates of E_{growth} at 6 months of age, than at 3½ months, partly due an extreme outlier in the data-set (but excluding this outlier did not change the conclusion) and partly due to increased variability in factors influencing TEE, e.g. physical activity energy expenditure, which was not determined as separate variables in the present study. Subsequently, errors between predicted and determined energy requirements were larger at 6 months reducing the power to detect any bias as significant.

Some biological day-to-day variation is accounted for, when TEE is measured as an average over 7 days, when using the DLW method. However, there are also methodological issues compromising the precision of the measurement of TEE, particularly when the study is conducted as a field study (section 4.4.4)¹⁵. For instance, administration of DLW to infants reduced the precision of the method, because dose administration was compromised (relative to children and adults), when infants did not comply with the procedure. This increased the individual errors on the measurement of TEE.

Therefore, although the factorial approach is considered the criterion method for determining infant energy requirements, when used in the field setting under free-living conditions it may still only be valid at the group level.

The First-Feed study was not powered according to the study design to show sex differences in infant energy balance variables, although these might have been significant if the sample had been larger. When comparing variables between groups of different sexes using independent t-test, there would be no study power advantage, as there was when studying differences in variables over time using repeated measurements (paired t-test). Butte and colleagues found consistent differences between the sexes in energy balance and body composition variables across the age range of 3 - 24 months^{145,260}, using the more precise multi-component model⁴⁴³, where the present study used the two-component model. Thus while we were underpowered to reveal differences between sexes as significant, we did find consistent trends in the differences between sexes.

The infants in the present study were living in a relatively affluent environment. Both socio-economic status within a country, as well as provenance in general might influence energy requirements and limit the ability of the present study to enable generalisations to be made. Infant energy requirements may be very different in developing countries where environments can be more challenging. For example, in Brazil, infants of lower socio-economic status families had 24% higher TEE, mainly caused by higher activity energy expenditure, and hence energy requirements, than infants from higher socio-economic status families⁴⁴⁴.

The present sample of infants was unique in the sense that the infants were exclusively breast-fed to 6 months as defined by WHO². Therefore variation in data caused by feeding mode was reduced in the present study, and such data has not been published previously. Only a few studies have attempted to determine energy requirements using the factorial approach in breast-fed infants: 7 studies in infants recovering from malnutrition, and 7 studies in pre-term infants²⁵².

6.5 Summary

In summary, energy balance variables of exclusively breast-fed infants were presented, and TEE was predicted fairly accurately in exclusively breast-fed infants at the group level by using the equation by FAO/WHO/UNU⁷.

The shortfall in energy supply during exclusive breast-feeding at 6 months as suggested in the Reilly-Wells hypothesis could not be replicated in the present study, when energy requirements were determined using the factorial approach. Prediction equations for energy requirements underestimated energy requirements at 3½ months of age at a level which is clinically important, compared to determined energy requirements using the criterion method (the factorial approach). Prediction equations for both TEE and infant energy requirements had errors which were too large for them to predict TEE or energy requirements with confidence at an individual level.

CHAPTER 7 BREAST-FEEDING PRACTICES

7.1 Breast-feeding from a behavioural perspective

The present chapter explores behavioural aspects of the Reilly-Wells hypothesis and some of these results were published in one of the papers generated from the First-Feed study¹⁶. The previous chapters in the present thesis revealed that infants in the present study, who were successfully breast-fed to around 6 months of age, had normal growth, adequate energy intakes, and milk intakes which were higher than literature values³. Hence, there did not seem to be any physiological constraints against breast-feeding exclusively for 6 months in the present sample. However, breast-feeding is a behavioural activity, which is strongly influenced by socio-demographic (section 1.1.5 and Chapter 3) and behavioural factors, some of which may be modifiable, for instance through appropriate breast-feeding education and support.

The behavioural mechanisms around infant feeding practices are very complex. Some studies have indicated that infant temperament and behaviour could predict later (toddler) body fatness^{352,445}. The idea would be that infant temperament and behaviour provoke the mother to certain responses in terms of feeding practices, which then affect infant energy balance⁴⁴⁶. Additionally, infant behaviour is subjectively perceived by the mother, and hence maternal temperament, perceptual framework, resources and coping strategies also affect this model^{209,447}. Although there is much behavioural research into the dynamics of these behavioural interactions, it is beyond the scope of the present thesis to discuss these issues in depth. However, a few approaches were included in the First-Feed study for the purpose of elucidating how exclusive breast-feeding to 6 months is possible from a behavioural perspective.

7.1.1 The Reilly-Wells hypothesis on breast-feeding practices

In Chapter 5 it was revealed that one in which way exclusive breast-feeding could be adequate to fulfil infant energy needs to 6 months of age was through high milk intakes, which increase over time. However, this result was obtained from well-supported mothers with a high degree of motivation to breast-feed.

Reflecting on the Reilly-Wells hypothesis⁴, it could be argued that since the mothers in the present study were not a representative sample, they may exhibit a breast-feeding behaviour which is not representative either. Given their high motivation to breast-feed, they may be spending more time and resources on breast-feeding and for instance allowing their infant to consume high volumes of milk due to either very frequent breast-feeds and/or long durations of breast-feeds.

Evidence reports that one of the most common reasons for cessation of exclusive breast-feeding is a perceived insufficient milk supply^{6,209,448,449}. If breast-feeding practices are perceived to be characterised by very frequent breast-feeds or long durations of breast-feeds, this could be a very crude marker for the perception of insufficient milk supply. Similarly, if milk intake is only adequate when infants are breast-fed very often or for extensive periods of time, it could explain why many mothers might struggle to manage exclusive breast-feeding to 6 months, in the context of a contemporary Western society. Therefore, it was an important issue for the present study, to include a measure of the maternal perception of her breast-feeding practices as well as the actual breast-feeding behaviour (recorded by the mother) in terms of frequency and duration of breast-feeds.

7.1.2 Breast-feeding problems

Practical breast-feeding problems also contribute to the risk of early cessation of exclusive breast-feeding²⁰⁹ or early cessation of any breast-feeding⁴⁵⁰. Breast-feeding problems are very common - particularly at the time of initiation and particularly for primiparous mothers⁴⁵⁰. If the present sample of 70% primiparous mothers did not experience any of these typical problems, this sample would be heavily biased in a way which could positively affect their ability to succeed with exclusive breast-feeding to 6 months, and thus diminish the ability of the present study to be generalised to other populations - not in terms of lactational physiology, but because behavioural constraints present in the general population was not prevalent in the present sample. Therefore, as the present sample was successful in exclusive breast-feeding, it was important to characterise the mothers' reports of breast-feeding problems.

7.1.3 Aims of the present chapter

The aims of the present chapter were:

- To explore maternal perceptions of breast-feeding practices as reported frequency and duration of breast-feeds.
- To characterise actual breast-feeding behaviour as recorded frequency and duration of breast-feeds.
- To explore changes over time in perception of breast-feeding practices, and compare those to actual adaptations in breast-feeding behaviour.
- To characterise mothers' reports of breast-feeding problems at initiation, and at each of the two time-points during the First-Feed study.

7.2 Methods

7.2.1 Perceived breast-feeding practices

As part of the First-Feed study, the mothers completed a 26-item breast-feeding questionnaire (section 2.6.2 and Appendix A.10), adapted from the IFPS II³⁶¹, regarding their perceived breastfeeding practices. The questionnaire was completed at the 1st and 2nd time-points as well as at the mid-way visit (20 weeks) and at the follow-up visit (52 weeks). The questions analysed for the present chapter are displayed in Table 7.1. As breast-feeding behaviour was only recorded at the 1st and 2nd time-points, only data from these time-points will be included in the present thesis.

Table 7.1 Items from the breast-feeding questionnaire regarding breast-feeding practices

Perceived breast-feeding practices	Question	Response options
Perceived frequency of breast-feeds	"In the past 7 days, how often was your baby fed breast milk per day?"	Line free to write any number. If the response was a range, a mean value was taken.
Perceived duration of breast-feeds	"About how long does an average breast-feed last?"	Response in categories: < 10 min 10 – 19 min 20-29 min 30-39 min 40-49 min > 50 min

7.2.2 Recorded breast-feeding behaviour

On days 3 - 5 during the week of measurement at each time-point, the mothers completed the BBD, as described in section 2.6.1 (Appendix A.12). The data from the diary were used as a record of actual breast-feeding behaviour, in terms of frequency and duration of breast-feeds. Frequency of breast-feeds was calculated as an average number of breast-feeds per 24 hrs of the three days. Duration of breast-feeds was calculated both as an average of total time spent on breast-feeding per 24 hrs, and as an average time spent per breast-feed.

7.2.3 Reported breast-feeding problems

From the background questionnaire, described in section 2.2.4 (Appendix A.4), information on initial breast-feeding problems was obtained retrospectively at Visit 1 around 15 weeks of age (Table 7.2). Additionally, information on current breast-feeding problems was obtained at each time-point.

Table 7.2 Items from the background questionnaire regarding perceived breast-feeding problems

Breast-feeding problems	Response options
<i>Initial breast-feeding problems:</i> “Did you have any of the following problems breast-feeding your baby during the first 2 weeks of breast-feeding?”	<i>Option to tick one or more of the following:</i> <ul style="list-style-type: none"> - My baby had trouble sucking or latching on - I didn’t have enough milk - My baby choked - My nipples were sore, cracked, or bleeding - My baby wouldn’t wake up to breast-feed regularly enough - My breasts were overfull (engorged) - My baby was not interested in breast-feeding - I had a thrush infection of the breast - My baby got distracted - I had a clogged milk duct - My baby breast-fed too often - My breasts were infected or abscessed - It took too long for my milk to come in - My breasts leaked too much - I had trouble getting the milk flow to start - I had some other problem - My baby didn’t gain enough weight or lost too much weight - I had no problems
<i>Current breast-feeding problems?</i> “Have you had any problems with breast-feeding since last visit?”	<i>Option to tick “yes” or “no”.</i>
“If yes, please describe...”	<i>Open-ended question...</i>

7.2.4 Data-handling and statistical methods

Data from the breast-feeding questionnaire and from the diaries were summarised as described in section 2.7.2. Changes between the 1st and 2nd time-points were tested using paired t-tests, or Wilcoxon signed rank test when not normally distributed, as well as χ^2 -test for the categorical variable of perceived duration of breast-feeds. Additionally, questionnaire and diary data were compared to explore differences between perceived breast-feeding practices and actual breast-feeding behaviour. Reported initial and current breast-feeding problems were described in frequencies (%) of the sample.

7.3 Results

7.3.1 Numbers of data-points

The participation was described in section 3.3.1. From the background questionnaire, data on initial breast-feeding problems were available from 50 mothers, and for current breast-feeding problems data were available from 47 mothers at each of the 1st and 2nd time-points. Furthermore, one mother forgot and one mother declined to fill in the breast-feeding questionnaire at the 2nd time-point. Hence, there were 47 and 45 breast-feeding questionnaires available for analysis at the 1st and 2nd time-points, with paired data for 43 mother-infant pairs.

As the BBD was completed during the DLW measurement, it was omitted if dose administration had been unsuccessful. At the 1st and 2nd time-points, there were four and three unsuccessful dose administrations, respectively. At the 2nd time-point, one mother declined to keep the diary and two mothers omitted to keep the diary, due to travelling and illness in the family, respectively. Therefore, there were 43 and 41 baby behaviour diaries available for analysis with paired data for 37 mother-infant pairs.

7.3.2 Frequency of breast-feeds

Data on feeding frequencies and duration of breast-feeds are summarised in Table 7.3. The median reported feeding frequency, as perceived by the mother, was 8 feeds/24 hrs at the 1st time-point and 7 feeds/24 hrs at the 2nd time-point.

Table 7.3 Breast-feeding practices as perceived in questionnaires and recorded in diaries.

	1 st time point	n	2 nd time point	n	P ^a	n
Questionnaire data:		47		45		
Average duration of a feed:					<0.001	
< 10 min/feed	6		11			
10 – 19 min/feed	15		16			
20 – 29 min/feed	16		13			
30 – 39 min/feed	6		4			
40 – 49 min/feed	2		1			
> 50 min/feed	2		0			
Perceived feeding frequency, feeds/24 hrs	8 (5 - 12)	44 ^b	7 (4 - 20)	44 ^c	>0.05	39
BBD data:	8 (5 - 15)	43	9 (5 - 18)	41	>0.05	38
Feeding frequency, feeds/24 hrs						
Duration of breast-feeds, min/24 hrs	173 (70 - 335)	43	143 (75 - 293)	41	<0.001	38
Mean duration per feed, min/feed	20 (6 - 46)	43	16 (8 - 33)	41	0.002	38

The variables were not normally distributed and are presented as median (minimum – maximum) or frequency, as appropriate.

^a Differences between time-points (χ^2 -test for questionnaire data and Wilcoxon signed rank test for diary data).

^b For breast-feeding frequency, three mothers omitted answering this question at 1st time-point.

^c For breast-feeding frequency, one mother omitted answering this question at 2nd time-point.

The diary records also revealed a median recorded feeding frequency of 8 feeds/24 hrs at the 1st time-point, while it was 9 feeds/24 hrs at the 2nd time-point, which was not a significant change over time (paired change over time; Wilcoxon signed ranks test, $P = 0.154$). By comparing the perceived and the recorded feeding frequency for each time-point, it became evident that the mothers underestimated the feeding frequency significantly at both time-points (Wilcoxon signed ranks test; 1st time-point: $P = 0.004$, 2nd time-point: $P < 0.001$) (Figures 7.1 and 7.2). The differences between perceived and recorded number of breast-feeds ranged from an underestimate of 6 feeds per day to an overestimate of 4 feeds per day at the 1st time-point, and at the 2nd time-point it ranged from an underestimate of 9 feeds per day to an overestimate of 10 feeds per day. At the 2nd time-point, there was a wider distribution in both perceived and recorded breast-feeding frequencies than at the 1st time-point.

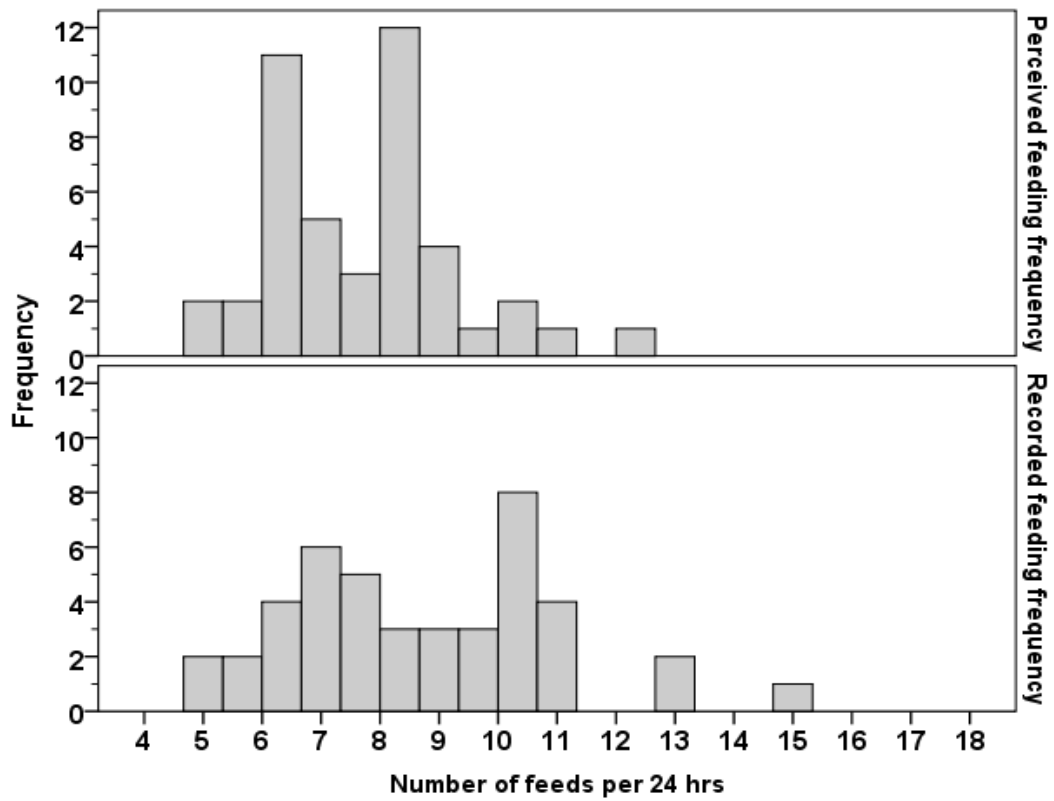


Figure 7.1 Frequency of breast-feeds as they were perceived and recorded at the 1st time-point.

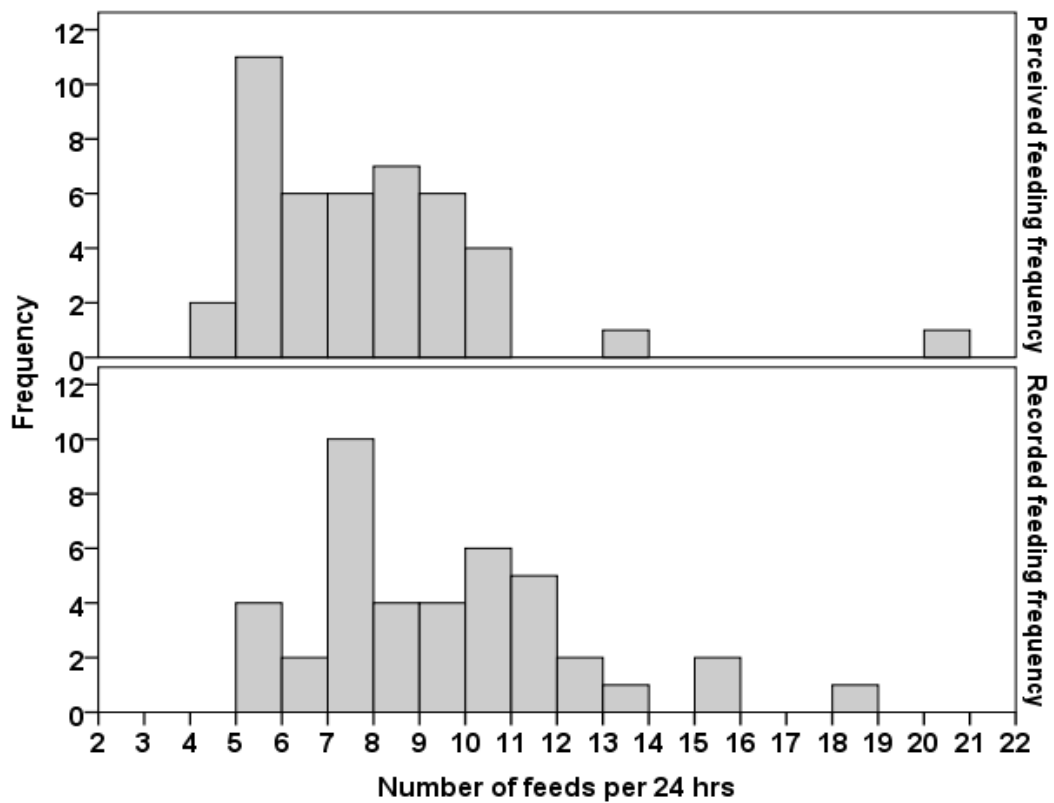


Figure 7.2 Frequency of breast-feeds as they were perceived and recorded at 2nd time-point.

7.3.3 Duration of breast-feeds

From the questionnaire data, the perceived average duration of a feed was reported across all categories. In all, 37 out of 47 mothers (79%) thought the average duration of a feed was under ½ hour at the 1st time-point, and this increased to 40 out of 45 mothers (89%) at the 2nd time-point. From the diary records, 32 out of 43 mothers (74%) recorded the average duration of a feed as less than 30 minutes at the 1st time-point, while 40 out of 41 mothers (98%) did this at the 2nd time-point. The recorded median (min - max) duration of a feed was 20 (6 - 46) min at the 1st time-point, and this decreased significantly to 16 (8 - 33) minutes at 2nd time-point (paired change over time; Wilcoxon signed rank test, $P = 0.002$).

7.3.4 Initial and current breast-feeding problems

Only 8% (4 mothers) reported not having any breast-feeding difficulties during the first 2 weeks post-partum (Figure 7.3).

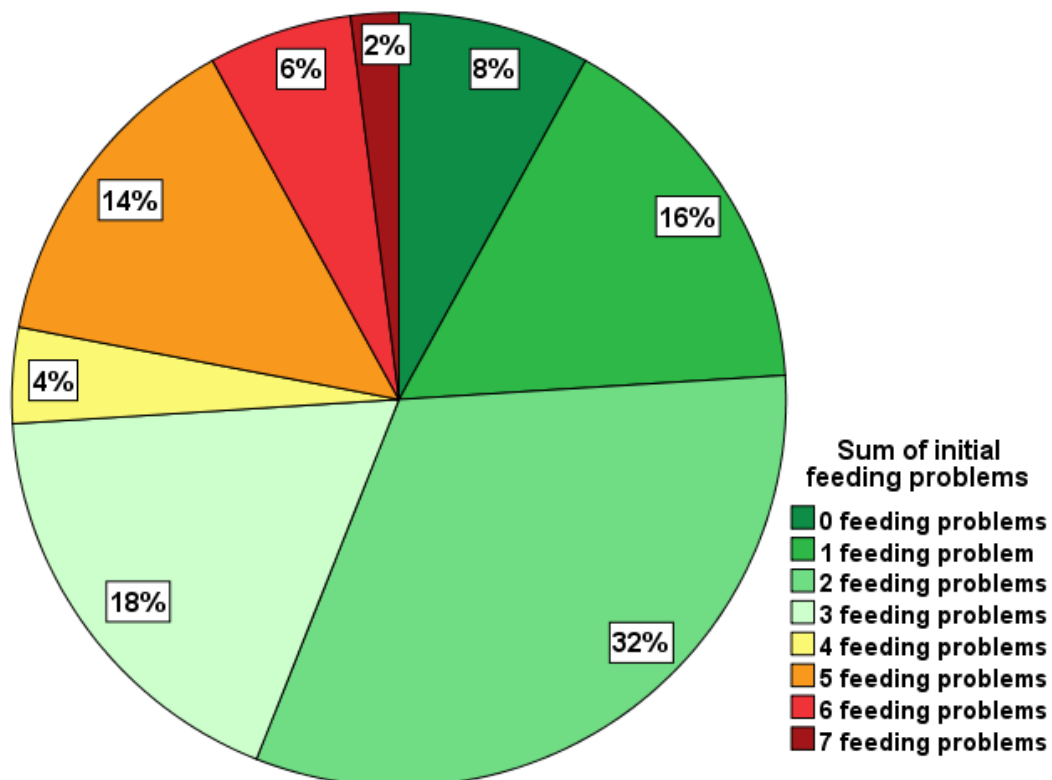


Figure 7.3 Sum of initial breast-feeding problems reported by the mothers (n=50).

Twenty-three mothers (56%) reported one, two or three problems during initiation. The most commonly reported problems were “My nipples were sore, cracked, or bleeding” (30, 60%), “My breasts were overfull (engorged)” (26, 52%), “infant having troubles sucking or latching on” (20, 40%), and “My baby wouldn’t wake up to breast-feed regularly enough” (13, 26%). At the 1st time-point, five out of 49 mothers reported still having one or more breast-feeding problems. One of those was excluded from the study. At the 2nd time-point, again five out of 47 mothers reported having one or more problems with breast-feeding, and two of those five also had problems at the 1st time-point.

7.4 Discussion

7.4.1 Main findings

From the Reilly-Wells hypothesis it was predicted that the very high milk intake of exclusively breast-fed infants may necessitate very frequent and/or very long breast-feeds, which may be increasing over time. Furthermore, a maternal perception of very high feeding frequencies and/or very long breast-feeds could be taken as crude markers of the breast-feeding being strained.

We found that the frequency of breast-feeds did not change significantly over time, and the duration of breast-feeds decreased significantly over time, in spite of increasing milk intake at the group level. Furthermore, mothers often underestimated feeding frequency, and therefore there was no indication of strain on breast-feeding practices during exclusive breast-feeding to 6 months. However, there was a wide range in difference between perceived and recorded breast-feeding frequencies, particularly at the 2nd time-point, which may suggest that the breast-feeding pattern is very variable and/or changeable. Finally, initial breast-feeding problems were common in this sample of mothers who successfully managed to breast-feed exclusively to around 6 months, and around 10% of the mothers reported having breast-feeding problems during the study.

7.4.2 Breast-feeding practices

In the IFPS II (section 2.6.2), up to 1400 mothers answered the same questionnaire as employed in the present study, with self-reports of perceived frequency and duration of breast-feeds⁴⁵¹.

The IFPS II employed the questionnaire on a monthly basis from birth to 6 months followed by every 1½ months until 12 months of age. Unfortunately the results from the IFPS II were reported with data merged for exclusively breast-fed infants, infants fed breast milk and formula milk as well as infants fed breast milk and complementary foods, so the results were not directly comparable to the present study. In contrast to the First-Feed study, breast-feeding frequency declined with age, concurrent with the introduction of complementary foods, which was reported to happen at all ages.

With regards to durations of breast-feeds, the IFPS II revealed similar findings as in the present study, where a longer duration breast-feeds were reported most often in the early post-partum period and then declined with infant age, while the shorter duration of breast-feeds were reported more frequently with age⁴⁵¹. Results from the IFPS II therefore indicate that the duration of breast-feeds changes throughout the period of lactation. The First-Feed study found the same results over the period from 3½ to 6 months of age, and these results were confirmed as the recorded breast-feeding behaviour showed a significant decrease in both the total time spent on breast-feeding per 24 hours and in the duration of breast-feeds.

Together these changes in perceived breast-feeding practices and recorded breast-feeding behaviour may suggest an increase in the efficiency of breast-feeding - manifested as infants increasing milk intake whilst reducing the time spent on breast-feeding - which is continuous throughout the period of lactation. Breast-feeding efficiency does not seem to reach a plateau in early infancy, but improves continuously even after an initial period of being established. Moreover, this increase in efficiency of breast-feeding more than compensates for the increase in infant demand for milk and energy.

7.4.3 Breast-feeding problems

The present study found initial breast-feeding problems to be common, and even during the study, around 10% still experienced problems with breast-feeding at the two time-points. The IFPS II found that primiparous mothers were more likely to report problems - for instance with infant latch or sucking⁴⁵⁰; in the present sample 70% of the mothers were primiparous.

In the IFPS II, higher rates of several breast-feeding problems were also found, including perceived insufficient milk supply, among mothers from lower social status backgrounds; so within their large sample, there seemed to be a social gradient with regards to reports of breast-feeding problems⁴⁵⁰. The sample in the First-Feed was not large enough, and also too homogenous, for a similar analysis, but it is well-established that infant feeding choices, including durations of exclusive and any breast-feeding, are strongly associated with socio-demographic status (section 1.1.5), and this could in part be determined by the perception of breast-feeding problems.

7.4.4 Perceived insufficient milk supply.

The IFPS II was a large cohort study not selected for breast-feeding, where 1,323 mothers rated the relative importance of 32 reasons for stopping breast-feeding during the study period⁴⁵⁰. The perception of the infant not being satisfied by breast milk alone was consistently among the top three most important reasons for stopping breast-feeding - regardless of at what age breast-feeding was ceased. Other studies have also found the perception of insufficient milk supply to be a leading cause of cessation of breast-feeding^{448,449}.

This is very unfortunate, as it is common for mothers to perceive their milk supply to be low during lactation - even when infants seem satisfied and are growing normally^{452,453}. In studies of self-selected populations with well-supported women choosing to exclusively breast-feed for 3 - 4 months, <5% of the mothers had a milk supply (measured by test-weighing) too low to support adequate infant growth compared to growth references⁴⁵⁴⁻⁴⁵⁷.

A review of studies on perceived insufficient milk supply included 20 studies⁶, but only one of the studies explored associations with actual measurements of infant milk intake, and this study found no link between perceived insufficient milk supply and actual milk intake by the infant. Unfortunately, this study suffered from a poor design as perceived insufficient milk supply was not measured simultaneously with the measurements of milk intake⁴⁵⁸. This review also confirmed the existence of a social gradient in the perception of insufficient milk supply, and noted how the perception of insufficient milk supply seemed to be a predominant reason for both supplementing with formula and for stopping breast-feeding altogether⁶.

Hence it seems that a significant majority of mothers stop breast-feeding, or cease exclusive breast-feeding due to a perceived problem (insufficient milk supply), which is normal to experience during the period of lactation, and may have little to do with the adequacy of breast-feeding to fulfil infant demands for milk and energy.

7.4.5 Implications for breast-feeding support

Several publications have argued that the perception of insufficient milk supply is misguided^{6,451,459}. Shealy and colleagues have pointed out that there seems to be a mismatch between the advice often given on breast-feeding practices, which often relates to frequencies and durations of breast-feeds⁴⁵¹, and lactation physiology, which is determined by other factors, such as degree of breast evacuation during a feed and diurnal variations in infant demand for milk and energy^{460,461}. However, the issue of exclusivity may be important, if milk supply is driven by demand. Predominantly breast-fed infants may take enough milk to continue to stimulate milk supply, but if infants are topped up with formula, this formula will replace energy needs from breast milk leading to reduced demand, and therefore reduced supply^{422,423}.

The “Ready, Steady, Baby” book published by NHS Scotland⁴⁶² as part of the Scottish Government’s “Healthier Scotland Initiative” recommends “6 to 8 breast-feeds per 24 hrs”, and “at least 6 breast-feeds per 24 hrs”, but the evidence base for such recommendation is unclear. In the present study, three (6%) exclusively breast-fed infants at each time-point had only recorded 5 feeds/24 hrs, and 20 infants (40%) had recorded more than 8 feeds/24 hrs at the 1st time-point, whilst this was 22 infants (44%) at the 2nd time-point.

Prentice and colleagues compared lactation performance of affluent mothers from Cambridge, UK, with malnourished mothers of Keneba, Gambia⁴⁶³. They found milk intakes (measured by test-weighing) to be very similar in the two populations. A noticeable difference between the two populations was that while the British women only breast-fed 5 - 6 times per 24 hours, the Gambian women breast-fed 14 - 18 times per 24 hours⁴⁶³. Despite this, the Gambian mothers successfully managed a high degree of exclusivity for extended durations, perhaps because their breast-feeding expectations continuously matched the infants breast-feeding needs⁴⁶³.

This raises the concern of an *ecological fallacy*, where a population mean is used as a standard guide for the individual - in spite of the fact that variation may be wide, and that this population mean may only be an appropriate standard for a small fraction of the population. It could be that the recommendation of 6 - 8 feeds per 24 hrs is based on an average frequency derived from any number of observations or studies. However, publishing such recommendation based on averages - especially where the normal range is wide - may increase the risk of mothers stopping exclusive breast-feeding unnecessarily, if they are concerned about the adequacy of their breast-feeding whilst not breast-feeding according to the average frequency, even though their infant may be growing normally.

Similarly, it is common for health professionals to advise feeds of 10 - 15 minutes on each breast (20 - 30 minutes per breast-feed)⁴⁵¹, but if duration of breast-feeds in fact changes throughout the period of lactation, as was suggested by the present study and also in the IFPS II⁴⁵⁰, only few infants will ever feed as advised. All this may cause mothers to develop unnecessary concerns about their breast-feeding, when the infant is feeding either more or less frequently, or for shorter or longer durations, than advised, even though this is common and infant energy requirements are being met.

Finally, breast-feeding problems were common in the present sample, and this has been confirmed in other studies as well^{450,451}. In the “Ready, Steady, Baby” book, common breast-feeding problems seem to be portrayed as abnormal and the guidance is to consult health professionals if and when they occur. Although mothers should always be encouraged to seek support for any breast-feeding issues, it is important to give the mothers a realistic expectation of what to expect in terms of breast-feeding problems. It may be setting mothers up for failure to characterise common breast-feeding issues as abnormal, as it may only be the most resourceful and persistent mothers who will contact health care professionals for help. Mothers from more deprived backgrounds are less likely to seek support⁴⁶⁴, and are more likely to employ coping strategies that are detrimental for breast-feeding success (e.g. topping up with formula).

7.4.6 Limitations and strengths of the present study

The First-Feed study was not designed to explore breast-feeding behaviour in any great detail. A questionnaire and a diary were used as crude instruments to obtain data on breast-feeding practices as they were perceived and recorded by the mothers. However, both of these were self-administered by the mother and will therefore have an accuracy and precision, which reflects this fact.

Any study using questionnaires and relying on maternal reports includes a risk of responder bias. In the present study, the questionnaire on perceived breast-feeding practices was administered before the BBD. Therefore, it was not possible for the mothers to let their perception of breast-feeding practices be influenced by their records of breast-feeding behaviour. If the mothers overestimated the frequency and/or durations of breast-feeds, this could be taken as a very crude marker for perceived insufficient milk supply. However, in the present study, mothers were more often underreporting frequency of breast-feeds, when comparing their perceptions with what they subsequently recorded.

The background questionnaire on initial breast-feeding problems did not include any option for stating other problems that were not listed, and the responses were reported retrospectively with a risk of memory bias. However, for the present study these questions were largely used to explore whether there had been problems, or no; and responses on how many problems were present initially is perhaps more likely to be underestimated than overestimated.

The present study found initial breast-feeding problems to be common, and even during the study, around 10% still experienced problems with breast-feeding. This finding indicates that the mothers in the present study were not unique, in the sense that they were “lucky” not to have met any challenges during the breast-feeding period. However, the present sample was unique in that they were selected for their determination to breast-feed exclusively to 6 months, in a population, where this practice is rare. They were also recruited from breast-feeding support groups and therefore they could be characterised as a sample of resourceful and well-supported mothers.

7.5 Summary

The present chapter found no strain on maternally reported breast-feeding practices in terms of high breast-feeding frequencies and/or long durations of breast-feeds as a result of breast-feeding exclusively to 6 months of age. Instead, we found indications that breast-feeding increased in efficiency over time. Thus, in relation to the Reilly-Wells hypothesis, the present chapter does not support the prediction that exclusive breast-feeding to 6 months could be made adequate only during strained breast-feeding practices, e.g. with very high frequency of breast-feeds and/or very long duration of breast-feeds.

Finally, initial breast-feeding problems were common in the sample of mothers who volunteered for this study, and around 10% of the mothers (5 mothers at each time-point) reported having problems with breast-feeding, both at 3½ and at 6 months of age. Hence the present sample was not unique with regard to the challenges they had to cope with during exclusive breast-feeding. Instead, they were selected for a high motivation to breast-feed and they were well-supported in achieving breast-feeding success.

CHAPTER 8 DISCUSSION AND CONCLUSION

8.1 The Reilly-Wells hypothesis

The Reilly-Wells hypothesis was based on the systematic review by Reilly and colleagues³. This review used clearly defined study and methodology selection criteria to review the evidence of milk intake of exclusively breast-fed infants from the developed world³. The findings on mean milk intake and milk energy content were combined, and indicated a shortfall in the energy supplied from breast milk during exclusive breast-feeding compared to infant energy requirements at 6 months of age⁴. Based on the Reilly-Wells hypothesis, it was thus proposed that successful exclusive breast-feeding to 6 months of age would include one or more of the following; 1) infants who were small and/or growing slowly, 2) milk intakes and/or milk energy content that were higher than literature values, 3) energy requirements that were lower than reference values, and/or 4) breast-feeding practices strained by very frequent and/or very time consuming breast-feeds in order for exclusive breast-feeding to be adequate to fulfil infant energy requirements to 6 months of age. The Reilly-Wells hypothesis was backed up by evidence of a very low prevalence of exclusive breast-feeding to 6 months of age^{8,178} and studies suggesting that perceived insufficient milk supply is a leading cause for mothers to start supplementing with formula or to introduce complementary foods before 6 months of age^{6,11}.

8.1.1 Infant growth during exclusive breast-feeding

The Reilly-Wells hypothesis was tested in the First-Feed study. As presented in Chapter 3, infants who were exclusively breast-fed to around 6 months of age had, overall, normal size and growth compared to the WHO Child Growth Standards³⁶⁹, which prescribes infant growth during optimal conditions. However, there were small, but significant, differences from the WHO Child Growth Standards in both infant size and growth. For instance, infants in the present study generally had low skinfold z-scores, but these increased significantly over time. Similarly, the infants increased significantly in weight-for-length and BMI-for-age z-score during the study, and decreased significantly in length-for-age z-score during exclusive breast-feeding.

In the MGRS³⁷⁰, the infant feeding criteria were different from the present study. For instance, per protocol the infants had to be predominantly breast-fed for at least 4 months³⁷⁶. Furthermore, the report of the MGRS revealed a varying degree of compliance with these infant feeding criteria³⁷⁶. As feeding mode influences growth, this may in part explain the deviations found in the present sample relative to the WHO Child Growth Standards.

The recently published randomised controlled trial from Iceland⁵⁴ with infants exclusively breast-fed, as defined by WHO², to either 4 or 6 months, also found infant size and growth to deviate from the WHO Child Growth Standards. However, these infants also had a positive birth weight z-score, and also a higher birth weight than the present study. The Iceland study found no difference in anthropometry z-scores at 6 months between infants exclusively breast-fed until either 4 or 6 months of age. Therefore, the study supported the view that growth is adequate during exclusive breast-feeding to 6 months, but growth patterns were still significantly different from the WHO Child Growth Standards.

In the present study, only a few infants were identified as beyond ± 2.0 z-scores relative to the WHO Child Growth Standards, and all but one of those infants were identified at one time-point only. Therefore, it is unlikely that any of the infants in the present sample were growth faltering or at risk of malnutrition, but it can never be excluded that individual infants may have had suboptimal growth. Because there are only limited ways of taking into account the hereditary component of growth trajectories, the nutrition that results in optimal growth for the individual infant can not be determined³³⁷.

8.1.2 Lactation performance during exclusive breast-feeding

The First-Feed study tested the Reilly-Wells hypothesis in terms of milk intake, and found that milk intakes were significantly higher than those found in the systematic review by Reilly and colleagues³ at 3½ and 6 months of age (Chapter 5). Furthermore, the First-Feed study found a significant increase in milk intake over time, particularly for boys, which is in contrast to the existing longitudinal evidence in exclusively breast-fed infants, although these studies generally used test-weighing and did not last until 6 months of age³.

The findings of the present study, which were different from the existing literature, could in part be explained by issues with methodology and study design (sections 5.4.2 and 5.4.3). For instance, Reilly and colleagues noted that many studies did not make sufficient corrections to data obtained by test weighing - a methodology criticised for underestimating milk intake (section 5.4.2). Milk intake from test-weighing studies needs to be corrected for IWL and metabolisability, before it is compared to references for energy requirements. The correction for metabolisability is based on a single study³³⁶ in newborn infants, while metabolisability may increase with age (section 5.4.5).

Other evidence of milk intake

The recent study by Da Costa and colleagues addressed the issues of metabolisability and underestimation by test-weighing by pooling data from studies that used the deuterium dose-to-mother method for measuring milk intake²⁷⁰. The deuterium dose-to-mother method is as 'objective' as the DLW method and avoids the additional imprecision caused by the dose-to-infant procedure (Chapter 4). They found milk intakes at 6 months of age around 800 g/d, but included very diverse populations and also predominantly breast-fed infants²⁷⁰. It was difficult to ascertain what the metabolisable milk intake would have been if the data were to be limited to exclusively breast-fed infants, as defined by WHO², from the developed world. Furthermore, the recent randomised trial from Iceland also used deuterium dose-to-mother, and found milk intakes of around 900 g/d⁵⁴ at 6 months of age. This is lower than the First-Feed study and in line with the systematic review by Reilly and colleagues³.

8.1.3 Infant energy requirements during exclusive breast-feeding.

The First-Feed study tested the Reilly-Wells hypothesis in terms of infant energy requirements (Chapter 6). At the group level, infants in the present study had an appropriate TEI at 6 months compared to references for energy requirements by FAO/WHO/UNU⁷, and the shortfall in energy supply during exclusive breast-feeding identified in the Reilly-Wells hypothesis could not be replicated when TEI was determined using the factorial approach. Conversely, at 3½ months of age, the infants had a TEI 8% higher than mean energy requirements, which is an overconsumption that is clinically important.

Differences in energy requirements due to feeding mode

The data for the FAO/WHO/UNU references⁷ on mean energy requirements were obtained using the criterion method of TEE added to an estimate of E_{growth} , which was the same method as was used in the present study. Even though, the references on mean energy requirements took into account four studies observing different growth patterns in breast-fed and formula-fed infants^{242,260,364,465}, these infants were not exclusively breast-fed as currently defined and recommended by WHO^{1,2}. Thus, it may be that current references for infant energy requirements do not sufficiently adjust for the different growth patterns and energy balance in exclusively breast-fed infants.

Furthermore, when comparing mean TEI with mean energy requirements, it is not possible to identify if any and how many infants get too much or too little. In theory, when mean intake equals mean requirements and distributions are identical, it is still possible (but unlikely) for 50% of a population to be fed inadequately⁴⁶⁶. Even when the mean energy intake from breast milk is only 90% of the mean energy requirements, as identified by Reilly and Wells⁴, and the distributions do not coincide, it is still possible for some infants to be overfed and others to be underfed. Comparing mean values (and overall distributions) can not reveal any information about adequacy at the individual level.

Additionally, when Reilly and Wells compared TEI calculated based on values in this systematic review with mean references for energy requirements⁴, they used the 50th percentile body weights according to the UK 1990 growth reference²⁹⁶ as a multiplier of body weight to obtain a mean value of energy requirements according to FAO/WHO/UNU⁷. This revealed an average shortfall in energy supply during exclusive breast-feeding of 6% and 13% for boys and girls, respectively (section 1.3.1). However, the UK 1990 reference was based on a population of infants where only a few would be exclusively breast-fed to 6 months of age²⁹⁶. Therefore using this method to derive mean energy requirements may not reflect a realistic mean value for energy requirement for exclusively breast-fed infants - when feeding mode affects infant energy requirements - although at the time that the Reilly-Wells hypothesis was proposed, the WHO Child Growth Standards were not yet published, and therefore no better growth reference would have been available for this comparison.

Since the Reilly-Wells hypothesis, the WHO Child Growth Standards have been published³⁸², where the 50th percentile weight-for-age of boys and girls are 7.9 kg and 7.3 kg, respectively, at 6 months of age. Using the WHO Child Growth Standards instead thus reduces the identified average shortfall in energy supply to 1.2% and 5% for boys and girls, respectively. The WHO Child Growth Standards may be a more appropriate growth reference for exclusively breast-fed infants, for reasons discussed in section 3.4.3, but even for carefully generated growth standards based on high quality anthropometric measurements, the basic assumption is that the observed growth reflects a normal growth under optimal conditions and a growth which is conducive to long-term health. In other words, any growth measured in apparently healthy infants and the energy requirements associated to fulfil such growth will at most only be representative for growth under the circumstances in which it was measured.

Performance of methods to predict energy requirements

References for mean energy requirements are intended to be a prescriptive reference at the group level to support and maintain health and good nutritional status⁷. However, in clinical practice, references for energy requirements per kg body weight are often used as a simple multiplier to derive individual energy requirements based on body weight alone. However, this is again an ecological fallacy, and the present study found this practice to be associated with very large errors at the individual level. These errors were negatively correlated with energy requirements, suggesting that the risk of underestimation was greater when energy requirements were high.

Just like using an appropriate reference for growth when growth monitoring exclusively breast-fed infants (section 3.4.3), it is important to have appropriate energy requirement references for exclusively breast-fed infants, as both over- and under-feeding can have long-term consequences for health⁴⁶⁷. Additionally, given the individual variations and errors in both measurements of TEI and in predictions of energy requirements (sections 6.4.3 and 6.4.4) any discrepancy between them at any given point in time, may not be representative of a true over- or under-consumption. Therefore, prediction equations to be used at the individual level could include a Standard Error of the Estimate, which could be used to calculate a range of acceptable energy intakes, rather than one mean value, based on the prediction for the individual infant.

Within this range, infant appetite could determine the amount of energy consumed, and energy intake could be assessed regularly to ascertain that the infant overall receives an adequate energy intake compared to energy requirements. Finally, when references or prediction equations are used as part of a treatment regime, indications of sufficient growth should always be ascertained, as this remains the most sensitive indicator of whether infant energy requirements are being met.

The ‘wisdom of crowds’ approach, prescribes the combining of several independently derived prediction equations in order to produce a more accurate prediction for use at the individual level. For equations to predict children’s body composition and adult resting metabolic rate, this has been demonstrated as a useful approach with lower mean bias as well as lower errors at the individual level⁴⁴⁰. However, more prediction equations for infant energy requirements are needed, before this approach can become useful in clinical practice.

8.1.4 Breast-feeding practices during exclusive breast-feeding

As described in Chapter 7, the First-Feed study also explored breast-feeding from a behavioural perspective. The notion was that even if the energy shortfall identified in the Reilly-Wells hypothesis was explained as milk intakes higher than literature values, it could be that breast-feeding practices were strained in order to achieve adequate milk output during exclusive breast-feeding to 6 months. Strained breast-feeding could be manifested as very frequent and/or very time consuming breast-feeding practices, which would be unfeasible for the typical mother in a contemporary Western society.

The present study found that breast-feeding practices adapted to become more efficient during exclusive breast-feeding between 3½ and 6 months of age, and that the time spent on breast-feeding decreased with increasing infant age. Furthermore, the present study also found that mothers underestimated the frequency of breast-feeds - hence their perception of their breast-feeding practices did not show obvious signs of being perceived as strained, although this was measured very crudely. The median breast-feeding frequency was 8 - 9 breast-feeds per 24 hours, but with a wide variation (section 7.4.2).

Finally, the present study found that common breast-feeding problems, which are often stated as reasons for ceasing exclusive (or any) breast-feeding and supplementing with formula or complementary foods⁴⁵⁰, were also prevalent in the present sample of mother-infant pairs. However, the present sample of mothers was fortunate in that they were recruited from infant feeding clinics and breast-feeding support groups, i.e. an environment that was very conducive to overcoming breast-feeding problems and achieving successful exclusive breast-feeding.

For comparison, the study by Prentice and colleagues showed that malnourished Gambian mothers achieved high milk outputs during exclusive breast-feeding through very frequent breast-feeds, which they sustained successfully for extended durations⁴⁶³. Prentice and colleagues provided further arguments to support the suggestion that it is behavioural aspects of maternal determination and motivation as well as appropriate support, rather than lactation physiology per se, which holds the key to successful exclusive breast-feeding⁴⁶³. The potential mechanisms by which such breast-feeding behaviour may influence lactation physiology, to make it adequate to cover infant energy needs, are still unclear.

8.2 Adequacy of exclusive breast-feeding to 6 months

8.2.1 Adequacy of breast-feeding as a behavioural issue

In the First-Feed study, 41 out of 50 mothers (82%) were successful in exclusive breast-feeding to around 6 months. However, these mothers were recruited from supportive environments and for their motivation to breast-feed, and the First-Feed study does not confirm that the breast-feeding practice displayed in the present study is normal and possible for all mothers. Therefore, to make the case for its possibility, it is necessary to search for arguments in the literature.

Variations in incidence and prevalence of breast-feeding

Referring to section 1.1.4, the incidence and prevalence of breast-feeding is very variable between countries and within countries, and has shown considerable historical changes in terms of decline and rise in prevalence over fairly short periods of time¹⁰.

These differences between populations of mothers that are likely to be very similar with regards to lactation physiology, may indicate that the barrier to breast-feeding success is not so much the lactation physiology per se, but may be more related to breast-feeding behaviour. For instance, the initiation, exclusivity and duration of breast-feeding are much higher in Scandinavian countries than in the UK¹⁸⁰, in spite of the populations' physiologically being very similar.

Furthermore, high levels of breast-feeding prevalence and exclusivity have to be preceded by high initiation rates, as mothers who do not initiate breast-feeding after birth are unlikely to initiate breast-feeding later on, unless they are subjected to intensive intervention⁴⁶⁹⁻⁴⁷¹. For mothers who do not initiate breast-feeding, insufficient lactation physiology (inadequate milk output) can not be the cause of breast-feeding attrition.

Breast-feeding prevalence responding to promotion initiatives

Since great effort is invested in the promotion of breast-feeding world-wide, the literature on this is vast, and it is beyond the scope of the present thesis to give an account of those efforts. However, a few selected relevant initiatives and reviews will be included here.

Firstly, studies finding that the Baby Friendly Hospital Initiative³⁴ (section 1.1.2) have been successful in improving initiation rates of both any and exclusive breast-feeding have been widely reported^{35,37,389,468-470}. Secondly, several recent reviews^{20,471,472}, systematic reviews^{36,473-477} and meta-analyses^{478,479} have been useful in elucidating how and why some promotion interventions have been more successful than others in improving breast-feeding initiation, duration and exclusivity. But the fact, that studies have reported the prevalence of breast-feeding to be responsive to promotion interventions^{51,86}, suggests that the issue with breast-feeding is one of adequate management and support, rather than an issue of physiologically inadequate milk supply.

Physiological indications of supply following demand

The physiology of lactation is well elucidated, and the mechanisms of hormonal regulation and neural reflex pathways necessary for the maintenance of milk synthesis and milk release are well known⁴⁸⁰.

Furthermore, a previous study demonstrated how milk output can adapt to increases in demand during breast-feeding⁴²¹, and very high milk outputs by mothers breast-feeding twins and triplets have been documented⁴⁸¹. The mechanisms by which exclusive breast-feeding to 6 months of age could be adequate are clearly possible, but the evidence suggesting that it is 'normal' for all mothers to produce enough milk to fulfil infant energy requirements to 6 months of age is still unclear.

8.2.2 The perception of insufficient milk supply

In spite of the WHO recommendation and the promotional efforts invested in breast-feeding, prevalence of exclusive breast-feeding at 6 months is low in many parts of the world, and a leading cause of ceasing exclusive breast-feeding is a maternally perceived insufficient milk supply. As discussed in section 7.4.4, no connection has been demonstrated between maternally perceived insufficient milk supply and actual inadequate infant milk intake in healthy growing infants⁶, but formula supplementation can be helpful in clinical cases of failure to thrive⁴⁸². The discussion in Chapter 7 went on to highlight a couple of areas in which the common guidance on breast-feeding practices is not consistent with the evidence of breast-feeding practices in the present study, and suggests that such discrepancy between the guidance given and what can be considered normal breast-feeding practices may be detrimental for breast-feeding success.

Misguided perception of insufficient milk supply

In section 1.1.5, it was briefly described how the theory of planned behaviour suggests that the mother's perception of her own breast-feeding, is an important factor in her decisions regarding infant feeding^{198,209}. Therefore, it remains a crucial issue to understand why and how mothers perceive their breast-feeding to be insufficient.

One study exploring the maternal perception of insufficient milk supply has characterised it as being based upon infant cues of satisfaction or insufficient weight gain⁴⁸³. The latter is important as growth remains the best indicator of whether infant energy requirements are met. However, a review of studies on insufficient milk supply revealed that mothers often rely on infant satisfaction alone as the indicator of adequate milk supply and also report insufficient milk supply in the neonatal period where the milk supply, is not yet established⁶.

Infant behaviours like fussiness, crying and wakefulness may be interpreted as dissatisfaction with the milk supply when they are just normal infant behaviours. A recent study in infant feeding and temperament found that breast-fed infants were rated as being more ‘difficult’ than formula-fed infants and suggested that this issue could be helped by supporting mothers in having a more realistic expectation of normal infant behaviour⁴⁸⁴.

Finally, there is the related issue of transient lactational crisis, which is characterised by a maternally perceived sense of “too little milk” that subsides after a few days and up to a week⁴⁵². Cohort studies have reported transient lactational crisis as common, and often initiated by external factors causing maternal stress or fatigue, but successful management is possible through appropriate support^{452,485}.

8.2.3 The World Health Organization recommendation

The WHO’s global recommendation of exclusive breast-feeding to 6 months of age (exactly), rather than 4 - 6 months¹, implies that exclusive breast-feeding to 6 months is adequate for all infants⁴⁶⁶. The present study was not of a size and representativeness that it could demonstrate that exclusive breast-feeding to 6 months of age is possible for all mothers. It merely demonstrates how highly motivated mothers do manage to achieve exclusive breast-feeding to around 6 months of age.

However, even within this relatively homogenous group of mothers, variables of milk intake, infant energy balance, and infant size and growth were very variable. Furthermore, milk intake was only very weakly related to infant age. The wide variation (particular biological variation) in such variables begs the question of how one particular point in time (i.e. 6 months of age) should be the appropriate duration of exclusive breast-feeding for all infants, when all other variables show such high variability.

The barriers against successful exclusive breast-feeding to 6 months of age, including practical breast-feeding problems and maternally perceived insufficient milk supply, do not appear to be related to the adequacy of exclusive breast-feeding. Therefore, they should be tackled through appropriate support and promotions that help to change cultural attitudes to breast-feeding, and this work is already under development in many places.

However, there may be valid reasons for introducing complementary foods at an earlier age than 6 months (exactly) for the individual infant, and the WHO recommendation does not seem to be flexible enough to accommodate this. Anecdotally, one mother in the present study called me and wanted to move forward the 2nd time-point of measurement as she felt that her boy was ready for complementary foods. At 22 weeks of age, he had a body weight of 9 kg, he was sitting without support and was grabbing for the mother's plate at the dinner table. The mother did not sense that she could not satisfy him with breast milk alone, but when evaluating his motor skills and level of maturity, it was obvious why the mother thought it was time to introduce him to complementary foods.

The argument that one simple global recommendation from the WHO sends a stronger message and may be more feasible for member countries to adopt, seems to be contrasted by the fact that it is achieved by so few, and that recent change in breast-feeding prevalence is small⁸. Mothers may not achieve this recommendation for behavioural reasons rather than due to any limitations in their lactation performance, and this is where much work remains to be done. However, there may be appropriate reasons for introducing complementary foods before 6 months of age, and, for instance, accommodating the biological variability in this recommendation by incorporating considerations to infant size, behavioural cues and developmental stage may improve the recommendations, even if they are more complex to implement.

8.3 Limitations and strengths of the First-Feed study

In any research study, it is important to appreciate the results in the light of the design and methodologies used to obtain them. As the limitations and strengths of the present study were considered in detail in each of the result chapters, the present section will only raise a certain points.

Design and methodology

The choice of design and methodology is often constrained by resources: Financial limits, time limits, and/or a question of man power. The present study was relatively small, as all measurements were performed by one researcher, the author. Therefore it was underpowered to look at details such as differences between sexes in energy balance variables.

The necessary inclusion criteria of exclusive breast-feeding resulted in the sample being unrepresentative of the population from which it was recruited. Therefore, the ability of the present study to enable generalisations to the rest of the population or indeed to any population should be considered with caution. However, it was a considerable strength over the existing literature that the present study was longitudinal and lasted to 6 months. Furthermore, it used a more objective method (the DLW method), than much of the existing literature, which is based on test-weighing, to measure milk intake and simultaneously measured variables of infant energy balance.

Imprecision of measurements

In the First-Feed study, anthropometric measurements were assessed for imprecision, and the dose-to-infant procedure for the DLW method was considered for the imprecision it added to the DLW method (Chapters 3 and 4). As such, the results on infant growth should be interpreted in the light of the imprecision of anthropometric measurements (sections 3.3.7 and 3.4.4). However, it is probably not unusual in a field study to have an imprecision in anthropometric measurements like the present study. More likely, the MGRS could be regarded as having a higher standard than is common due to the rigorous study protocol.

The imprecision caused by the dose-to-infant procedure of the DLW method as well as other analytical aspects of this method, may have contributed to the variability in data on milk intake and infant energy balance variables. However, the DLW method remains the gold standard for measuring energy balance variables and in the present study it was even further improved by having simultaneous measurements of milk intake.

Inaccuracy of measurements

It is possible that the anthropometric measurements were biased, perhaps due to insufficient training. There is no way of ascertaining this, but imprecision in the anthropometric measurements alone would not explain why the infants deviated significantly in z-score from the WHO Child Growth Standards at the group level, and similar results have been found in other infants⁵⁴ who were breast-fed as defined and recommended by WHO^{1,2}.

On the other hand, bias in anthropometric measurements would perhaps produce results that were significantly different from zero, but would not account for the significant change in z-scores over time. Therefore, the results in growth were unlikely to be due to measurement error and bias alone, but could at least in part be explained by differences in feeding practices between the MGRS and the present study. There is also the possibility, that milk intake and variables of infant energy balance in the present study have been overestimated. In the present study, the approach to avoiding systematic bias in the variables derived from the isotopes was to correct the spills that were accounted for (Chapter 4). Although such corrections are used in many areas of research, a similar approach has not been described in other isotope studies¹⁵.

8.4 Directions for future research

It is a great improvement to the existing literature to have systematic guidelines to follow, when reporting results of a research study. The present study was conducted and reported in accordance with the “Strengthening the Reporting of Observational Studies in Epidemiology” (STROBE) Statement⁴⁸⁶, and it is the hope that future studies will adopt this approach as well. In addition, having consensus on breast-feeding definitions - particularly the definition of exclusive breast-feeding² - greatly improves comparability between studies and therefore future studies should adhere to these definitions as well as specifying any circumstances or cases in which there were deviations from these definitions.

More recent studies do improve the evidence base on milk intake^{54,270}, but given the issues of study design and methodology (sections 5.4.2 and 5.4.3), it would be desirable to obtain more evidence on milk intake in infants breast-fed as defined and recommended by WHO^{1,2}. Such a study should be large, multi-centred, longitudinal, and use isotopic methods to determine milk intake. A separate issue also worth exploring is the metabolisability of breast milk and whether it changes over time (section 5.4.5).

As has been discussed in sections 3.4.3 and 6.4.3, growth varies with feeding mode and particularly between formula-fed and breast-fed infants, and since exclusive breast-feeding is now recommended for 6 months, there is a great need to expand the evidence base on both TEI/energy requirements and E_{growth} in exclusively breast-fed infants.

A further improvement would be to employ the four-component model for determining body composition and the composition of growth. It would also be interesting to replicate the present study with a better study power and under different socio-economic circumstances in order to be able to take such circumstances into account in references for energy requirements in exclusively breast-fed infants.

Finally, the extensive research into the behavioural issues of breast-feeding attrition and breast-feeding success needs to continue until it forms a firm evidence base on which to build new and even more effective promotion initiatives, so that, when mothers cease exclusive breast-feeding, they do so for the right reasons.

8.5 Conclusions

In summary, the present thesis took as its basis the Reilly-Wells hypothesis and was designed as a longitudinal observational study to test the hypotheses, that for exclusive breast-feeding to be adequate to fulfil infant energy requirements to 6 months of age, it would necessitate that 1) the infants were small and/or growing slowly, 2) milk intakes and/or milk energy content were higher than literature values, 3) energy requirements were lower than reference values, and/or 4) breast-feeding practices were strained by very frequent and/or very time consuming breast-feeds. The present study recruited a sample of highly motivated mothers and found that the infant growth was normal relative to WHO Child Growth Standards, milk intakes were higher than literature values and increased over time, total energy intakes were higher than reference energy requirements at 3½ months and similar at 6 months of age, and breast-feeding practices did not show signs of being strained, but increased in efficiency over time. Therefore, the present study suggests that mothers can manage exclusive breast-feeding to 6 months of age, by being motivated and having appropriate support, and by making adaptations that are both physiological (increased lactation performance) and behavioural (adapted breast-feeding practices).

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APPENDICES

Appendix A: Documents used in the First-Feed study

A.1 Information sheet and invitation to participate

A.2 Data Sheet 1: Eligibility and oral information

A.3 Informed Consent Form

A.4 Data Sheet 2: Background information

A.5 Data Sheet 3: Maternal anthropometry

A.6 Data Sheet 4: Infant anthropometry

A.7 Data Sheet 5: Dose administration

A.8 Data Sheet 6: Urine sampling

A.9 Urine sample instructions

A.10 Breast-feeding practices questionnaire

A.11 Rothbart's Infant Behaviour Questionnaire

A.12 Baby Behaviour Diary

A.13 Data Sheet 7: Readiness for complementary foods

A.14 Complementary feeding questionnaire

A.1 Information sheet and invitation to participate



Information Sheet and Invitation to Participate The FirstFeed Study

The First-Feed study is a research study on breast-feeding for mothers with babies from about 3 to 6 months old. **You are being invited to take part in this research study.** If you have a baby about 13 weeks or younger, are exclusively breast-feeding, and would like to continue exclusive breast-feeding until your baby is 6 months old, we would like you to read on.

Before you decide whether to take part or not, it is important for you to understand why the research is being done, and what it will involve. Please take time to read the following information carefully. Talk to others about the study, if you wish. If you have any questions about the study, we are happy to answer them without any obligation for you to participate.

Background and purpose of the study

The World Health Organisation recommends that mothers breast-feed their babies exclusively (giving no other food or water) until their babies are 6 months old. Breast-feeding indisputably has many advantages for mother and infant, but there is a lack of knowledge about whether all mothers can provide their babies with enough energy from breast milk for 6 months. In addition, for those mothers who do successfully breast-feed their babies exclusively for 6 months it is not known *how* they do so (e.g. do they increase the amount of breast-milk they provide, or increase the calorie content of their milk, or both?).

Therefore in this research study we want to investigate how it is possible for mothers who are breast-feeding exclusively to satisfy their baby's energy needs up to the time of 6 months old. We would use the information we get from the study to help make recommendations to promote breast-feeding in future.

Why have I been chosen?

If you have a baby of 13 weeks or younger, that you are breast-feeding, then you might be able to participate in this study. It is entirely voluntary whether you choose to take part- you are under no obligation to take part and if you take part you are free to withdraw from the study at any time. If you decide not to take part, this will not affect any care you or your baby receive.

*What are we going to do?***Measures of calorie intake by babies and their calorie needs**

The measures we want to make on babies will allow us to assess how much milk mothers are transferring to their babies, the calorie content of the milk, the calorie needs of the babies, and whether babies seem satisfied by the amount of calories being provided by their mothers' milk.

If you agree to take part in this study we would give your baby about 15-20 ml of sterilised water which contains two harmless and non-radioactive substances (deuterium and oxygen-18) which are present naturally in all individuals including your babies. Over one week we would measure how fast these substances appear in the urine of your baby and this is how we calculate how much breast milk your baby gets and how much energy is in that breast milk. We would do the first measurement when your baby is about 4 months old and the second measurement when he/she is about 6 months old, if you are still exclusively breast-feeding by this time. If you decide to start giving your baby solids before 6 months, we would like to do the second measurement either just before you introduce the solids or at 6 months while asking you to record any solids eaten during the week of measurements, or even at both occasions, if you are okay with it.

The urine samples will be collected from your baby by putting small cotton wool balls in the nappy, which are then collected after urination and compressed to obtain the urine sample. These methods have been used successfully and safely before in many previous studies of babies.

Growth measurements of babies in the study, and measures on mothers

At about age 4 months and again at 6 and 12 months we would want to measure your baby's weight, length, knee-heel-length and two skinfold thicknesses (the amount of fat we can 'pinch' on your babies arm and back). At these times we would also want to measure your weight, height, skinfold thicknesses, and waist circumference and estimate your body fat using a special scale.

Measures of baby temperament and feeding patterns

When your baby is 4 months old and again when he/she is about 6 months old we will ask you to complete two short questionnaires which measure the temperament of your baby and your breast-feeding routines. In addition we will ask you to keep a very simple diary of the behaviour of your baby for 3 days when your baby is 4 and 6 months old.

When and where are we going to do these measures ?

Participating in this study will involve receiving **8 - 14 home visits**. So you don't have to go anywhere to participate - the researchers will come to you. Each visit will last for about 1½ hours. The visits are divided into **3 - 6 visits** during the first week of measurement (when your baby is 3 months old), **3 - 6 visits** during the last week of measurement (when your baby is 6 months old) plus one visit mid-way between 3 and 6 months. A final home visit will be made to you when your baby is about 12 months old.

This study does not involve any medical treatments and we do not want you to change the way you are feeding your baby for the study- it is an 'observational study'. This means that even if you enter the study, when your baby is about 3 months, but later decide not to breast-feed exclusively for 6 months, you can still participate in the study. We will then give you the option of either doing the second measurement when you decide to introduce formula or complementary feeding, or we can do the second measurement when your baby is 6 months, and then ask you to record any other intake than breast milk.

What is the outcome of these measures?

From the urine samples we collect from your baby we will measure their calorie expenditure, calorie intake and milk intake.

We will use the information we get from the study to help develop breast-feeding advice for mothers in future. At the end of the study we will give all mothers who took part a summary of our results, and the results from the measures we made on their baby if they wish.

What are the risks, benefits and inconveniences for me and my baby?

There are no significant risks to you or your baby. Both the deuterium and oxygen-18 we use, and the measurements of growth, are safe. There will be no benefits to taking part for you or your baby, other than the special attention you will receive on the breast-feeding and the close monitoring of your baby's growth. You will also be given detailed feedback on the results of the study, which we hope will benefit future breast-feeding mothers in Scotland and elsewhere.

Participating in the study will involve receiving numerous visits from us, for which you and your baby have to be home. We will try to minimise this inconvenience by arranging the visits at the time of the day that suits you best. Your baby will have to have the nappy changed some extra times in order to obtain the urine samples. For this inconvenience we will provide you with replacement nappies. We would also like to give you a little present for your baby (worth <£5 each) upon completion of the study to thank you for your participation.

What about confidentiality?

When you are entered in the study, you will be given a StudyID - a unique number - which will then be used on all subsequent files with information about you and your family. This StudyID will appear instead of name and other personal details on all subsequent files, samples and data. All papers will be kept in a locked cupboard in a locked office at Yorkhill Hospitals. All electronic data will be kept on pass-word protected PC's.

What if I want to participate or to discuss the study further?

Please contact us by using the phone numbers or email addresses given below.

If you have any complaints about the study please contact Professor Lawrence Weaver, Professor of Child Health at the University of Glasgow and Yorkhill Hospitals (Tel.: 0141-201-0235).

Who has checked and approved this study ?

The study has been funded by the Scottish Executive Health Department Chief Scientist's Office.

The study has been approved by the local NHS Ethics Committee and by the local NHS Research and Development Office.

The study will be part of the degree of Doctor of Philosophy (Ph.D.) for the researcher (Susan Bjerregaard Nielsen) at the University of Glasgow.

Contact details:

Susan Bjerregaard Nielsen

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A.2 Data Sheet 1: Eligibility and oral information

Eligibility and contact information

10 Questions concerning inclusion/exclusion criteria:

1) Are you giving your baby breast milk only? Yes..... ☐ No..... ☐

(if no; exclude from participation)

2) Have you ever given your baby anything else than breast milk?

Yes..... ☐ No..... ☐

If Yes; How old was your baby? _____Weeks (exclude if older than 6 weeks)

What did you give your baby (formula/water/other)? _____

How much/how often? _____

For what reason did you feed your baby other than breast milk?

3) Do you intend to try and keep exclusive breast-feeding until your baby is 6 months or older? (if no; exclude) Yes..... ☐ No..... ☐

4) Did you give birth to twins? (if yes, exclude) Yes..... ☐ No..... ☐

5) When was your baby due? (dd/mm/yyyy) Period:_____

UL:_____

6) When was your baby born? (dd/mm/yyyy) DOB:_____

Calculate week of gestation at birth (exclude if <37 or >42 weeks):

GA: _____

(i.e. check if DOB is more than 21 days before due date)

7) Baby's birth weight? _____grams or _____ - _____ pounds/ounces

1 pound = 453.6 grams, 1 ounce = 28.3 grams

Check >2500 grams or >4 pounds, 2 ounces

8) Have you/your baby had any illness during pregnancy/after delivery?

Yes..... ☐ No..... ☐

Yes; which; _____ (Exclude if relevant for infant metabolism)

9) Have you/your baby had any conditions affecting the breast-feeding?

Yes..... ☐ No..... ☐

Yes; which; _____ (Exclude if likely to re-occur)

10) Are you involved in other research studies? Yes..... ☐ No..... ☐

If Yes, determine whether they might interfere with breast-feeding or our study in general.

Contact information

Mother's full name: _____

DOB: ____/____/____ Age: ____years

Father's full name: _____

DOB: ____/____/____ Age: ____years

Baby's full name: _____

DOB: ____/____/____ (Question 6 above)

Address: _____

Postcode: _____

Email: _____

Tel.: _____ Preferred time to be contacted: _____

Mobile: _____

Arrangements for visits are made: Yes (See data-sheet 1) No

Waiting for potential participant to contact us before _____ (date of baby turning 15 weeks)

A.3 Informed Consent Form



Informed Consent Form

The FirstFeed Study

Information given by _____

Please initial boxes below:

I confirm that I have read and understand the FirstFeed Information Sheet (November 2007, version 4) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

☐

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

☐

I understand that relevant sections of any of my personal information and data collected during the study, may be looked at by responsible individuals from the FirstFeed Study, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

☐

I am aware that the results of this study will be published and that data will be anonymised for these purposes.

☐

I agree to take part, and let my baby take part, in the above study.

☐

Name of participant Date Signature

Researcher Date Signature

A.4 Data Sheet 2: Background information



Background Information

Sections:

1. Delivery
2. Initial breast-feeding
3. Parents' education and employment
4. Family information
5. Previous growth measurements
6. Health information - visit 0
7. Health information - visit 6
8. Health information - visit 7
9. Health information - visit 12

1. Delivery

1. Birth weight: _____grams or _____ - _____ pounds, ounces

(data-sheet 1, Question 7)

2. Birth length: _____cm (inches; 1 inch = 2.54 cm)

3. Head circumference: _____cm

4. Gestation weeks at birth: _____weeks (data-sheet 1, Question 6)

2. Initial breast-feeding

5. After delivery about how long after your delivery did you breast-feed or try to breast-feed your baby for the very first time?

Less than 30 min... ☐ 3 to 6 hours... ☐ 1 day... ☐

30 to 60 min... ☐ 7 to 12 hours... ☐ 2 days... ☐

1 to 2 hours... ☐ 13 to 24 hours... ☐ >2 days... ☐

6. During the first few days after your baby was born, did you feed him or her...

- ☐ Whenever he or she cried or seemed hungry
- ☐ Sometimes on a schedule AND sometimes when he or she cried or seemed hungry
- ☐ On a schedule or routine

7. How long did it take for your milk to come in?

- <1 day... ☐ 2 days... ☐ 3 days... ☐ 4 days... ☐
- >4 days... ☐

8. Did you have any of the following problems breast-feeding your baby during your first 2 weeks of breast-feeding? (PLEASE "X" ALL THAT APPLIES)

- My baby had trouble sucking or latching on..... ☐
- I didn't have enough milk..... ☐
- My baby choked..... ☐
- My nipples were sore, cracked, or bleeding..... ☐
- My baby wouldn't wake up to breast-feed regularly enough..... ☐
- My breasts were overfull (engorged)..... ☐
- My baby was not interested in breast-feeding..... ☐
- I had a thrush infection of the breast..... ☐
- My baby got distracted..... ☐
- I had a clogged milk duct..... ☐
- My baby breast-fed too often..... ☐
- My breasts were infected or abscessed..... ☐
- It took too long for my milk to come in..... ☐
- My breasts leaked too much..... ☐
- I had trouble getting the milk flow to start..... ☐
- I had some other problem..... ☐
- My baby didn't gain enough weight or lost too much weight..... ☐
- I had no problems..... ☐

9. Were you given information about any breast-feeding support groups or services before delivery or before you went home from hospital or birth centre?

Yes...☐ No...☐

10. Since your baby was born, have you attended a breast-feeding class or breast-feeding support group?

Yes...☐ No...☐

3. Education and employment

11. Level of education:

Mother: standard / higher grades / college / university

Father: standard / higher grades / college / university

standard-grades; lower level; 14 years (compulsory)

higher-grades; additional 1-2 years (5. - 6. years)

College and/or university (b.sc. degree, M.sc./Ph.D. higher degree)

12. Occupation:

Mother: _____ If unemployed →Go to Q17

Father: _____

12b. Approximately annual pretax household income (in whole £1.000): _____

13. How many months pregnant were you when you stopped working?

☐ I stopped working before I became pregnant

☐ Less than 3 months pregnant

☐ 3 to 5 months pregnant

☐ 6 to 7 months pregnant

☐ 8 to 9 months pregnant

☐ Did not stop working before the birth

14. How old will your baby be, when you are due back from maternity leave?
_____weeks

15. What will you do with your baby while you are working? (PLEASE "X" ALL THAT APPLIES)

- ☐ My baby is cared for by a family member
- ☐ I keep my baby with me while I work outside my home
- ☐ My baby is cared for by someone not in my family
- ☐ I keep my baby with me while I work from home

16. When you are due back at work, or if you are working right now; do you work for -

- ☐ The same number of hours as before pregnancy
- ☐ Reduced hours
- ☐ Not working after delivery

4. Family information

17. What's your marital status:

- ☐ Married
- ☐ Living with partner
- ☐ Divorced
- ☐ Single

18. How many siblings does your baby have: _____

(include half-siblings, if they live with you and your baby)

Age of siblings: _____

How many babies have you had: _____

19. On the average, how many cigarettes do you smoke a day (if not, write 0)?

20. Have you had gestational diabetes with this pregnancy?

Yes...☐ No...☐ I don't know...☐

21. About how much weight did you gain during pregnancy? _____kg/pounds

5. Previous growth measurements of the infant (from health visitor/GP):

Date: _____ Length: _____ Weight: _____

Date: _____ Length: _____ Weight: _____

Date: _____ Length: _____ Weight: _____

Date: _____ Length: _____ Weight: _____

Date: _____ Length: _____ Weight: _____

Date: _____ Length: _____ Weight: _____

6. Health information - Visit 1

22. Which of the following problems did your baby have during the past 2 weeks?

(PLEASE "X" ALL THAT APPLIES)

- | | |
|---|--|
| <input type="checkbox"/> Fever | <input type="checkbox"/> Runny nose or cold |
| <input type="checkbox"/> Diarrhoea | <input type="checkbox"/> Respiratory Syncytial Virus (RSV) |
| <input type="checkbox"/> Vomiting | <input type="checkbox"/> Cough or wheeze |
| <input type="checkbox"/> Ear infection | <input type="checkbox"/> Asthma |
| <input type="checkbox"/> Colic | <input type="checkbox"/> Food allergy |
| <input type="checkbox"/> Fussy or irritable | <input type="checkbox"/> Eczema (atopic dermatitis) |
| <input type="checkbox"/> Reflux | <input type="checkbox"/> None of these |

23. Did your baby receive any of the following medicines in the past 2 weeks?
(Please do not include vitamins or minerals.)

Antibiotics...☐

Other prescription medicines...☐

Non-prescription medicines...☐

24. Have you had any problems with breast-feeding during the past 2 weeks
(e.g. mastitis)?

Yes...☐ No...☐

If yes, please describe: _____

25. Do you give your baby vitamin supplements?

Which: _____

Dose: _____

7. Health information - Visit 4

26. Which of the following problems have your baby had since last visit?

(PLEASE "X" ALL THAT APPLIES)

- | | |
|---|--|
| <input type="checkbox"/> Fever | <input type="checkbox"/> Runny nose or cold |
| <input type="checkbox"/> Diarrhoea | <input type="checkbox"/> Respiratory Syncytial Virus (RSV) |
| <input type="checkbox"/> Vomiting | <input type="checkbox"/> Cough or wheeze |
| <input type="checkbox"/> Ear infection | <input type="checkbox"/> Asthma |
| <input type="checkbox"/> Colic | <input type="checkbox"/> Food allergy |
| <input type="checkbox"/> Fussy or irritable | <input type="checkbox"/> Eczema (atopic dermatitis) |
| <input type="checkbox"/> Reflux | <input type="checkbox"/> None of these |

27. Did your baby receive any of the following medicines since the last visit?
(Please do not include vitamins or minerals.)

Antibiotics...☐

Other prescription medicines...☐

Non-prescription medicines...☐

28. Have you had any problems with breast-feeding since the last visit (e.g. mastitis)?

Yes...☐ No...☐

If yes, please describe: _____

29. Do you give your baby vitamin supplements?

Which: _____

Dose: _____

8. Health information - Visit 5

30. Which of the following problems have your baby had since the last visit?

(PLEASE "X" ALL THAT APPLIES)

- | | |
|---|--|
| <input type="checkbox"/> Fever | <input type="checkbox"/> Runny nose or cold |
| <input type="checkbox"/> Diarrhoea | <input type="checkbox"/> Respiratory Syncytial Virus (RSV) |
| <input type="checkbox"/> Vomiting | <input type="checkbox"/> Cough or wheeze |
| <input type="checkbox"/> Ear infection | <input type="checkbox"/> Asthma |
| <input type="checkbox"/> Colic | <input type="checkbox"/> Food allergy |
| <input type="checkbox"/> Fussy or irritable | <input type="checkbox"/> Eczema (atopic dermatitis) |
| <input type="checkbox"/> Reflux | <input type="checkbox"/> None of these |

31. Did your baby receive any of the following medicines since the last visit?
(Please do not include vitamins or minerals.)

Antibiotics...☐

Other prescription medicines...☐

Non-prescription medicines...☐

32. Have you had any problems with breast-feeding since the last visit (e.g. mastitis)?

Yes...☐ No...☐

If yes, please describe: _____

33. Do you give your baby vitamin supplements?

Which: _____

Dose: _____

9. Health information - Visit 8

34. Which of the following problems have your baby had since the last visit?

(PLEASE "X" ALL THAT APPLIES)

- | | |
|---|--|
| <input type="checkbox"/> Fever | <input type="checkbox"/> Runny nose or cold |
| <input type="checkbox"/> Diarrhoea | <input type="checkbox"/> Respiratory Syncytial Virus (RSV) |
| <input type="checkbox"/> Vomiting | <input type="checkbox"/> Cough or wheeze |
| <input type="checkbox"/> Ear infection | <input type="checkbox"/> Asthma |
| <input type="checkbox"/> Colic | <input type="checkbox"/> Food allergy |
| <input type="checkbox"/> Fussy or irritable | <input type="checkbox"/> Eczema (atopic dermatitis) |
| <input type="checkbox"/> Reflux | <input type="checkbox"/> None of these |

35. Did your baby receive any of the following medicines since the last visit?
(Please do not include vitamins or minerals.)

Antibiotics...☐

Other prescription medicines...☐

Non-prescription medicines...☐

36. Have you had any problems with breast-feeding since the last visit (e.g. mastitis)?

Yes...☐ No...☐

If yes, please describe: _____

37. Do you give your baby vitamin supplements?

Which: _____

Dose: _____

A.5 Data Sheet 3: Maternal anthropometry

Maternal measurements



Visit: _____

Date: _____

Height:

measurement: _____mm

measurement: _____mm

measurement: _____mm

Subscapular:

measurement: _____mm

measurement: _____mm

measurement: _____mm

Triceps:

measurement: _____mm

measurement: _____mm

measurement: _____mm

Waist circumference:

measurement: _____mm

measurement: _____mm

measurement: _____mm

TANITA:	
Exercise for the past 48 hours; Yes/No	
Time since last meal or drink (approx. 2 hrs)	
Time since voiding (<30 min)	

NB: Note date and time on TANITA printout and attach

A.6 Data Sheet 4: Infant anthropometry**Infant anthropometric measurements**

Visit: _____

Date: _____

Weight of infant:

measurement: _____kg

measurement: _____kg

Knee-heel-length: (attach printout)

Skinfold thickness:

Subscapular:

measurement: _____mm

measurement: _____mm

measurement: _____mm

Triceps:

measurement: _____mm

measurement: _____mm

measurement: _____mm

Length:

measurement: _____cm

measurement: _____cm

measurement: _____cm

Notes:

A.7 Data Sheet 5: Dose administration



Isotope Dosing

Dose preparation:

Date of preparation: _____ Fridge Temperature: _____ °C

Weight of infant: _____ kg (data-sheet 4)

Required dose (2.6 g/kg BW x 1.2): _____ g

Dose sample for analysis: Yes / No

Weight of sealed bottle without dose: _____ g

Weight of sealed bottle with dose: _____ g

Weight of large bag, sealed bottle, filtered isotope, naso-gastric tube, sterile 20 ml syringe: _____ g - before dosing

Dosing:

Date of dosing: _____ Time of dosing: _____ Last feed @: _____

Weight of infant: _____ kg

Weight of large bag, sealed bottle, filtered isotope, naso-gastric tube, sterile 20 ml syringe: _____ g - after dosing

Difference: _____ g (X)

Tissues no: _____ Tissues no: _____ Tissues no: _____

Before: _____ g Before: _____ g Before: _____ g

After: _____ g After: _____ g After: _____ g

Difference: _____ g Difference: _____ g Difference: _____ g

Total spill: _____ g (Y)

Dose given (X-Y): _____ g

Comment:

A.8 Data Sheet 6: Urine sampling



Urine sample collection

Day -1: Before dosing

Date of predose 1: _____

Time dry: ____:____ ____:____ ____:____ ____:____ ____:____

Time wet: ____:____

Day 0: Before dosing

Time of dosing: ____:____

Time dry: ____:____ ____:____ ____:____ ____:____ ____:____

Time wet: ____:____

Day 1: Approximately 24 hrs after dosing (Check at least every ½ hour)

Time dry: ____:____ ____:____ ____:____ ____:____ ____:____

Time wet: ____:____

Day 2: Approximately 48 hrs after dosing (Check at least every ½ hour)

Time dry: ____:____ ____:____ ____:____ ____:____ ____:____

Time wet: ____:____

Day 3 - 5: Baby Behaviour Diary !

(Write down times for extra urine samples overleaf)

Day 6: Approximately 144 hrs (6 days) after dosing (Check at least every ½ hour)

Time dry: ____:____ ____:____ ____:____ ____:____ ____:____

Time wet: ____:____

Day 7: Approximately 168 hrs (7 days) after dosing (Check at least every ½ hour)

Time dry: ____:____ ____:____ ____:____ ____:____ ____:____

Time wet: ____:____

If your baby has had anything at all to eat or drink during this week of urine collection, please write it down here:

Time	Amount	Type	Regurgitation/vomit
____:____	_____	_____	_____
____:____	_____	_____	_____
____:____	_____	_____	_____

A.9 Urine sample instructions



Collecting urine samples - Instructions

We need to collect urine samples again another 5 times; today (day 0), tomorrow (day 1), day 2, day 6 and day 7.

The urine samples should **not** be collected as the first urination of the day (since this will have urine that have been produced overnight), but other than that you can choose any time of day that suits you. However, we would recommend that the samples be collected after the first nappy change in the morning, since that makes the rest of the day free of having to plan when to do it. It doesn't have to be at the exact same time of day, but it is important that you **record the time of urine collection as accurately as you can**. This should be written down on the data-sheet provided.

Take the cotton wool from the dated re-sealable plastic bag, and put them "strategically positioned" in the clean nappy. Record the time this was done on the data-sheet (**Time dry**).

Check the nappy regularly and if the cotton wool is still dry, record the time of checking under **Time dry**. It is not important how much time lapses between checks, as long as it is **within 30 minutes** and you record the exact time of check. Keep doing so, until the cotton wool seems wet. Our experience is this need to be repeated 2-4 times.

Record the time, when the cotton wool is wet on the data-sheet (**Time wet**).

Put the cotton wool in the provided 20 ml syringe and put the plunger back in place.

Squeeze the urine into the 5 ml sample tube provided. Please try and collect as much urine as possible, and fill the 5 ml sample tube provided (till the top of the label on the tube). The tube should be sealed carefully with the lid (make sure it's leak-tight) and put it back in the re-sealable bag, which should also be closed tight with as little air in it as possible.

Please store the 5 ml sample tube in its re-sealable bag in the freezer until next visit.

If you want, you can dispose of the cotton wool balls and clean and re-use the syringe if you like (for instance as a toy when the baby is bathing).

Many thanks for your help. If you have any problems or are in any doubt about the procedure, please don't hesitate to contact me at:

Tel: 0141 201 9341 (daytime)

Mob: 07876 214 464 (any time)

Email: s.nielsen.1@research.gla.ac.uk

Best Wishes

Susan

A.10 Breast-feeding practices questionnaire



Breast-feeding practices

A. Breast-feeding at present

1. Has your baby used a dummy in the past 7 days? Yes...☐ No...☐
2. During the past 7 days, how often was your baby put to bed with breast milk?
 - ☐ At most bedtimes, including naps
 - ☐ At most night bedtimes, but not naps
 - ☐ At most naps, but not night bedtimes
 - ☐ Only occasionally at bedtimes, including naps
 - ☐ Never
3. In the past 7 days, how often was your baby fed breast milk per day?

4. Does your baby usually feed from both breasts at each feeding?
Yes...☐ No...☐ Baby is only fed expressed milk...☐ **Go to Q9**
5. Does your baby usually let go of the breast him or herself?
 - ☐ Yes, both breasts
 - ☐ Yes, first breast only
 - ☐ Yes, second breast only
 - ☐ No
6. About how long does an average breast-feed last?

Less than 10 minutes... <input type="checkbox"/>	20 to 29 minutes... <input type="checkbox"/>	40 to 49 minutes... <input type="checkbox"/>
10 to 19 minutes... <input type="checkbox"/>	30 to 39 minutes... <input type="checkbox"/>	>50 minutes... <input type="checkbox"/>
7. In an average 24-hour period, what is the LONGEST time for you, the mother, between breast-feeds or expressing milk? Please count the time from the start of one breast-feeding or expressing session to the start of the next.
 _____ HOURS AND _____ MINUTES

8. Since your baby was born, have you ever expressed or tried to express milk?

Yes, but I got no milk...☐ Yes, and I got milk...☐ No...☐ → **Go to Q14**

9. How old was your baby the first time you expressed or tried to express milk?

_____ DAYS OR _____ WEEKS

10. How many times in the past 7 days was your baby fed expressed breast milk to drink? _____ TIMES, IF 0 → **Go to Q14**

11. How often does your baby drink all of his or her cup or bottle of expressed milk?

Never...☐ Rarely...☐ Sometimes...☐ Most of the time...☐ Always...☐

12. How often is your baby encouraged to finish a cup or bottle if he or she stops drinking before the expressed breast milk is all gone?

Never...☐ Rarely...☐ Sometimes...☐ Most of the time...☐ Always...☐

13. For what reasons have you expressed milk in the past 7 days?

(PLEASE "X" ALL THAT APPLIES)

- ☐ To relieve engorgement
- ☐ Because my nipples were too sore to breast-feed
- ☐ To increase my milk supply
- ☐ To get milk for someone else to feed to my baby
- ☐ To mix with cereal or other food
- ☐ To have an emergency supply of milk
- ☐ To donate to a baby other than my own
- ☐ For me to feed to my baby when I do not want to breast-feed or when my baby cannot breast-feed
- ☐ To keep my milk supply up when my baby could not breast-feed

B. Breast-feeding in future

14. How old do you think your baby will be when you first feed him (or her) formula or any other food besides breast milk?

☐ 5 to 6 months ☐ 7 to 9 months ☐ More than 9 months

15. Do you plan to continue breast-feeding after you return to work?

Yes...☐ No...☐ Do not plan to work after the baby's birth...☐

C. Breast-feeding attitudes

16. Which of the following statements is closest to your opinion? The best way to feed a baby is:

- ☐ Breast-feeding
- ☐ A mix of both breast and formula feeding
- ☐ Formula feeding
- ☐ Breast-feeding and formula feeding are equally good ways to feed a baby

17. How strongly do you agree or disagree with the following statements?

STRONGLY	SOMEWHAT	NEITHER AGREE	SOMEWHAT	STRONGLY
AGREE...(1)	AGREE...(2)	NOR DISAGREE...(3)	DISAGREE...(4)	DISAGREE...(5)

Infant formula is as good as breast milk....

(1) ☐ (2) ☐ (3) ☐ (4) ☐ (5) ☐

If a baby is breast-fed, he or she will be less likely to get ear infections...

(1) ☐ (2) ☐ (3) ☐ (4) ☐ (5) ☐

If a baby is breast-fed he or she will be less likely to get a respiratory illness....

(1) ☐ (2) ☐ (3) ☐ (4) ☐ (5) ☐

If a baby is breast-fed he or she will be less likely to get diarrhoea....

(1) ☐ (2) ☐ (3) ☐ (4) ☐ (5) ☐

Babies should be exclusively breast-fed for the first 6 months....

(1) ☐ (2) ☐ (3) ☐ (4) ☐ (5) ☐

If a child was breast-fed, he or she will be less likely to become obese....

(1) ☐ (2) ☐ (3) ☐ (4) ☐ (5) ☐

18. Using 1 to mean “Very Uncomfortable” and 5 to mean “Very Comfortable,” how comfortable would you be in the following situations?

VERY UNCOMFORTABLE

VERY COMFORTABLE

(1)

(2)

(3)

(4)

(5)

Breast-feeding your baby in the presence of close women friends...

☐
☐
☐
☐
☐

Breast-feeding your baby in the presence of men and women who are close friends

☐
☐
☐
☐
☐

Breast-feeding your baby in the presence of men and women who are not close friends

☐
☐
☐
☐
☐

19. Did you breast-feed, for any time at all, any of your other babies?

Yes...☐

No...☐

This is my first...☐

D. Sleeping arrangements and other information

20. During the past 7 days, what was the longest time your baby slept at night without waking?

< 2 hours...☐ 3-4 hours...☐ 5-6 hours...☐ 7-8 hours...☐ >8 hours...☐

21. What are your reasons for bringing your baby to bed with you?

☐ It is commonly done in my family (“X” ALL THAT APPLIES)

☐ To bottle feed

☐ Sleeping with my baby helps the baby or me to sleep better

☐ To help with a blocked milk duct or other breast-feeding problem

☐ I think it is safer if my baby sleeps with me or us

☐ To be close or bond

☐ A doctor or nurse advised sleeping with my baby

☐ To comfort when fussy

☐ To breast-feed

☐ To comfort when sick

22. What are your reasons for not bringing your baby to bed with you?

(PLEASE "X" ALL THAT APPLIES)

- ☐ It is not commonly done in my family
- ☐ We waken each other up, or baby wakens me or others in the bed
- ☐ I think it is safer if my baby does not sleep with me or us
- ☐ I don't think the baby should sleep with me because I smoke, take sedative medicine or other reason
- ☐ A doctor or nurse advised not sleeping with my baby
- ☐ I think it will be too hard to get my baby to sleep in a crib when he or she is older

23. How many stools (dirty nappies) does your baby usually have in a 24-hour period? If less than one a day, how many days usually pass between stools?

_____ NUMBER OF STOOLS IN 24 HOURS

OR ONE STOOL EVERY _____ DAYS

24. How would you describe your baby's stool in the past 7 days?

Hard...☐ Formed...☐ Soft...☐ Semi-watery...☐ Watery...☐

25. How many teeth does your baby have now? (Write in 0 if none.) _____
NUMBER OF TEETH

26. During the past 7 days, have you had any health conditions which made it hard or impossible for you to take care of your baby? Yes...☐ No...☐

A.11 Rothbart's Infant Behavior Questionnaire



Infant Behavior Questionnaire - Revised⁴⁸⁷

Date: _____

Age: _____ weeks

INSTRUCTIONS: Please read carefully before starting:

As you read each description of the baby's behaviour below, please indicate how often the baby did this during the **LAST WEEK** (the past seven days) by circling one of the numbers in the left column. These numbers indicate how often you observed the behaviour described during the last week.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(X)
Never	Very Rarely	Less Than Half the Time	About Half the Time	More Than Half the Time	Almost Always	Always	Does Not Apply

The "Does Not Apply" (X) column is used when you did not see the baby in the situation described during the last week. For example, if the situation mentions the baby having to wait for food or liquids and there was no time during the last week when the baby had to wait, circle the (X) column. "Does Not Apply" is different from "Never" (1). "Never" is used when you saw the baby in the situation but the baby never engaged in the behaviour listed during the last week. For example, if the baby did have to wait for food or liquids at least once but never cried loudly while waiting, circle the (1) column.

Please be sure to circle a number for every item.

Feeding

During feeding, how often did the baby:

1 2 3 4 5 6 7 X (1) lie or sit quietly?

1 2 3 4 5 6 7 X (2) squirm or kick?

1 2 3 4 5 6 7 X (3) wave arms?

1 2 3 4 5 6 7 X (4) notice lumpy texture in food (e.g., oatmeal)?

In the last week, while being fed in your lap, how often did the baby:

1 2 3 4 5 6 7 X (5) seem to enjoy the closeness?

1 2 3 4 5 6 7 X (6) snuggle even after she was done?

1 2 3 4 5 6 7 X (7) seem eager to get away as soon as the feeding
was over?

How often did your baby make talking sounds:

1 2 3 4 5 6 7 X (8) while waiting in a high chair for food?

1 2 3 4 5 6 7 X (9) when s/he was ready for more food?

1 2 3 4 5 6 7 X (10) when s/he has had enough to eat?

Sleeping

Before falling asleep at night during the last week, how often did the baby:

1 2 3 4 5 6 7 X (11) show no fussing or crying?

During sleep, how often did the baby:

1 2 3 4 5 6 7 X (12) toss about in the crib?

1 2 3 4 5 6 7 X (13) move from the middle to the end of the crib?

1 2 3 4 5 6 7 X (14) sleep in one position only?

After sleeping, how often did the baby:

1 2 3 4 5 6 7 X (15) fuss or cry immediately?

1 2 3 4 5 6 7 X (16) play quietly in the crib?

1 2 3 4 5 6 7 X (17) cry if someone doesn't come within a few
minutes?

How often did the baby:

1 2 3 4 5 6 7 X (18) seem angry (crying and fussing) when you left
her/him in the crib?

1 2 3 4 5 6 7 X (19) seem contented when left in the crib?

1 2 3 4 5 6 7 X (20) cry or fuss before going to sleep for naps?

When going to sleep at night, how often did your baby:

1 2 3 4 5 6 7 X (21) fall asleep within 10 minutes?

1 2 3 4 5 6 7 X (22) have a hard time settling down to sleep?

1 2 3 4 5 6 7 X (23) settle down to sleep easily?

When your baby awoke at night, how often did s/he:

1 2 3 4 5 6 7 X (24) have a hard time going back to sleep?

1 2 3 4 5 6 7 X (25) go back to sleep immediately?

When put down for a nap, how often did your baby:

1 2 3 4 5 6 7 X (26) stay awake for a long time?

1 2 3 4 5 6 7 X (27) go to sleep immediately?

1 2 3 4 5 6 7 X (28) settle down quickly?

1 2 3 4 5 6 7 X (29) have a hard time settling down?

When it was time for bed or a nap and your baby did not want to go, how often
did s/he:

1 2 3 4 5 6 7 X (30) whimper or sob?

1 2 3 4 5 6 7 X (31) become tearful?

Bathing and Dressing

When being dressed or undressed during the last week, how often did the baby:

1 2 3 4 5 6 7 X (32) wave her/his arms and kick?

1 2 3 4 5 6 7 X (33) squirm and/or try to roll away?

1 2 3 4 5 6 7 X (34) smile or laugh?

1 2 3 4 5 6 7 X (35) coo or vocalize?

When put into the bath water, how often did the baby:

1 2 3 4 5 6 7 X (36) smile?

1 2 3 4 5 6 7 X (37) laugh?

1 2 3 4 5 6 7 X (38) splash or kick?

1 2 3 4 5 6 7 X (39) turn body and/or squirm?

When face was washed, how often did the baby:

1 2 3 4 5 6 7 X (40) smile or laugh?

1 2 3 4 5 6 7 X (41) fuss or cry?

1 2 3 4 5 6 7 X (42) coo?

When hair was washed, how often did the baby:

1 2 3 4 5 6 7 X (43) smile?

1 2 3 4 5 6 7 X (44) fuss or cry?

1 2 3 4 5 6 7 X (45) vocalize?

Play

How often during the last week did the baby:

1 2 3 4 5 6 7 X (46) look at pictures in books and/or magazines for
2-5 minutes at a time?

1 2 3 4 5 6 7 X (47) look at pictures in books and/or magazines for
5 minutes or longer at a time?

1 2 3 4 5 6 7 X (48) stare at a mobile, crib bumper or picture for
5 minutes or longer?

1 2 3 4 5 6 7 X (49) play with one toy or object for 5-10 minutes?

1 2 3 4 5 6 7 X (50) play with one toy or object for 10 minutes or
longer?

1 2 3 4 5 6 7 X (51) spend time just looking at playthings?

1 2 3 4 5 6 7 X (52) repeat the same sounds over and over again?

1 2 3 4 5 6 7 X (53) laugh aloud in play?

1 2 3 4 5 6 7 X (54) repeat the same movement with an object for
2 minutes or longer (e.g., putting a block in a
cup, kicking or hitting a mobile)?

1 2 3 4 5 6 7 X (55) pay attention to your reading during most of
the story when looking at picture books?

1 2 3 4 5 6 7 X (56) smile or laugh after accomplishing something
(e.g., stacking blocks)?

1 2 3 4 5 6 7 X (57) smile or laugh when given a toy?

1 2 3 4 5 6 7 X (58) smile or laugh when tickled?

How often during the last week did the baby enjoy:

1 2 3 4 5 6 7 X (59) being sung to?

1 2 3 4 5 6 7 X (60) being read to?

1 2 3 4 5 6 7 X (61) hearing the sound of words, as in nursery
rhymes?

1 2 3 4 5 6 7 X (62) looking at picture books?

1 2 3 4 5 6 7 X (63) gentle rhythmic activities, such as rocking or
swaying?

1 2 3 4 5 6 7 X (64) lying quietly and examining his/her fingers or
toes?

1 2 3 4 5 6 7 X (65) being tickled by you or someone else in your
family?

1 2 3 4 5 6 7 X (66) being involved in rambunctious play?

1 2 3 4 5 6 7 X (67) watching while you, or another adult,
playfully made faces?

1 2 3 4 5 6 7 X (68) touching or lying next to stuffed animals?

1 2 3 4 5 6 7 X (69) the feel of soft blankets ?

1 2 3 4 5 6 7 X (70) being rolled up in a warm blanket?

1 2 3 4 5 6 7 X (71) listening to a musical toy in a crib?

When playing quietly with one of her/his favorite toys, how often did your baby:

1 2 3 4 5 6 7 X (72) show pleasure?

1 2 3 4 5 6 7 X (73) enjoy lying in the crib for more than 5
minutes?

1 2 3 4 5 6 7 X (74) enjoy lying in the crib for more than 10
minutes?

When something the baby was playing with had to be removed, how often did
s/he:

1 2 3 4 5 6 7 X (75) cry or show distress for a time?

1 2 3 4 5 6 7 X (76) seem not bothered?

When tossed around playfully how often did the baby:

1 2 3 4 5 6 7 X (77) smile?

1 2 3 4 5 6 7 X (78) laugh?

During a peekaboo game, how often did the baby:

1 2 3 4 5 6 7 X (79) smile?

1 2 3 4 5 6 7 X (80) laugh?

How often did your baby enjoy bouncing up and down:

1 2 3 4 5 6 7 X (81) while on your lap?

1 2 3 4 5 6 7 X (82) on an object, such as a bed, bouncer chair, or
toy?

How often did the infant look up from playing:

1 2 3 4 5 6 7 X (83) when the telephone rang?

1 2 3 4 5 6 7 X (84) when s/he heard voices in the next room?

When your baby saw a toy s/he wanted, how often did s/he:

1 2 3 4 5 6 7 X (85) get very excited about getting it?

1 2 3 4 5 6 7 X (86) immediately go after it?

When given a new toy, how often did your baby:

1 2 3 4 5 6 7 X (87) get very excited about getting it?

1 2 3 4 5 6 7 X (88) immediately go after it?

1 2 3 4 5 6 7 X (89) seem not to get very excited about it?

Daily Activities

How often during the last week did the baby:

1 2 3 4 5 6 7 X (90) cry or show distress at a change in parents' appearance, (glasses off, shower cap on, etc.)?

1 2 3 4 5 6 7 X (91) when in a position to see the television set, look at it for 2 to 5 minutes at a time?

How often during the last week did the baby:

1 2 3 4 5 6 7 X (92) when in a position to see the television set, look at it for 5 minutes or longer?

1 2 3 4 5 6 7 X (93) protest being placed in a confining place (infant seat, play pen, car seat, etc.)?

1 2 3 4 5 6 7 X (94) startle at a sudden change in body position (for example, when moved suddenly)?

1 2 3 4 5 6 7 X (95) appear to listen to even very quiet sounds?

1 2 3 4 5 6 7 X (96) attend to sights or sounds when outdoors (for example, wind chimes or water sprinklers)?

1 2 3 4 5 6 7 X (97) move quickly toward new objects?

1 2 3 4 5 6 7 X (98) show a strong desire for something s/he wanted?

1 2 3 4 5 6 7 X (99) startle to a loud or sudden noise?

1 2 3 4 5 6 7 X (100) look at children playing in the park or on the playground for 5 minutes or longer?

1 2 3 4 5 6 7 X (101) watch adults performing household activities (e.g., cooking, etc.) for more than 5 minutes?

1 2 3 4 5 6 7 X (102) squeal or shout when excited?

1 2 3 4 5 6 7 X (103) imitate the sounds you made?

1 2 3 4 5 6 7 X (104) seem excited when you or other adults acted
in an excited manner around him/her?

When being held, how often did the baby:

1 2 3 4 5 6 7 X (105) pull away or kick?

1 2 3 4 5 6 7 X (106) seem to enjoy him/herself?

1 2 3 4 5 6 7 X (107) mold to your body?

1 2 3 4 5 6 7 X (108) squirm?

When placed on his/her back, how often did the baby:

1 2 3 4 5 6 7 X (109) fuss or protest?

1 2 3 4 5 6 7 X (110) smile or laugh?

1 2 3 4 5 6 7 X (111) wave arms and kick?

1 2 3 4 5 6 7 X (112) squirm and/or turn body?

When the baby wanted something, how often did s/he:

1 2 3 4 5 6 7 X (113) become upset when s/he could not get what
s/he wanted?

1 2 3 4 5 6 7 X (114) have tantrums (crying, screaming, face red)
when s/he did not get what s/he wanted?

When placed in an infant seat or car seat, how often did the baby:

1 2 3 4 5 6 7 X (115) wave arms and kick?

1 2 3 4 5 6 7 X (116) squirm and turn body?

1 2 3 4 5 6 7 X (117) lie or sit quietly?

1 2 3 4 5 6 7 X (118) show distress at first; then quiet down?

When frustrated with something, how often did your baby:

1 2 3 4 5 6 7 X (119) calm down within 5 minutes?

When your baby was upset about something, how often did s/he:

1 2 3 4 5 6 7 X (120) stay upset for up to 10 minutes or longer?

1 2 3 4 5 6 7 X (121) stay upset for up to 20 minutes or longer?

1 2 3 4 5 6 7 X (122) soothe her/himself with other things (such as
a stuffed animal, or blanket)?

When rocked or hugged, in the last week, how often did your baby:

1 2 3 4 5 6 7 X (123) seem to enjoy her/himself?

1 2 3 4 5 6 7 X (124) seemed eager to get away?

1 2 3 4 5 6 7 X (125) make protesting noises?

When reuniting after having been away during the last week how often did the
baby:

1 2 3 4 5 6 7 X (126) seem to enjoy being held?

1 2 3 4 5 6 7 X (127) show interest in being close, but resisted
being held?

1 2 3 4 5 6 7 X (128) show distress at being held?

When being carried, in the last week, how often did your baby:

1 2 3 4 5 6 7 X (129) seem to enjoy him/herself?

1 2 3 4 5 6 7 X (130) push against you until put down?

While sitting in your lap:

1 2 3 4 5 6 7 X (131) how often did your baby seem to enjoy
her/himself?

1 2 3 4 5 6 7 X (132) how often would the baby not be content
without moving around?

How often did your baby notice:

1 2 3 4 5 6 7 X (133) low-pitched noises, air conditioner, heating
system, or refrigerator running or starting up?

1 2 3 4 5 6 7 X (134) sirens from e.g. ambulances at a distance?

1 2 3 4 5 6 7 X (135) a change in room temperature?

1 2 3 4 5 6 7 X (136) a change in light, e.g. cloud over the sun?

1 2 3 4 5 6 7 X (137) sound of an airplane passing overhead?

1 2 3 4 5 6 7 X (138) a bird or a squirrel up in a tree?

1 2 3 4 5 6 7 X (139) fabrics with scratchy texture (e.g., wool)?

When tired, how often was your baby:

1 2 3 4 5 6 7 X (140) likely to cry?

1 2 3 4 5 6 7 X (141) show distress?

At the end of an exciting day, how often did your baby:

1 2 3 4 5 6 7 X (142) become tearful?

1 2 3 4 5 6 7 X (143) show distress?

For no apparent reason, how often did your baby:

1 2 3 4 5 6 7 X (144) appear sad?

1 2 3 4 5 6 7 X (145) seem unresponsive?

How often did your baby make talking sounds when:

1 2 3 4 5 6 7 X (146) riding in a car?

1 2 3 4 5 6 7 X (147) riding in a shopping cart?

1 2 3 4 5 6 7 X (148) you talked to her/him?

Two Week Time Span

When you returned from having been away and the baby was awake, how often did s/he:

1 2 3 4 5 6 7 X (149) smile or laugh?

When introduced to an unfamiliar adult, how often did the baby:

1 2 3 4 5 6 7 X (150) cling to a parent?

1 2 3 4 5 6 7 X (151) refuse to go to the unfamiliar person?

1 2 3 4 5 6 7 X (152) hang back from the adult?

1 2 3 4 5 6 7 X (153) never “warm up” to the unfamiliar adult?

When in the presence of several unfamiliar adults, how often did the baby:

1 2 3 4 5 6 7 X (154) cling to a parent?

1 2 3 4 5 6 7 X (155) cry?

1 2 3 4 5 6 7 X (156) continue to be upset for 10 minutes or
longer?

When visiting a new place, how often did the baby:

1 2 3 4 5 6 7 X (157) show distress for the first few minutes?

1 2 3 4 5 6 7 X (158) continue to be upset for 10 minutes or more?

1 2 3 4 5 6 7 X (159) get excited about exploring new
surroundings?

1 2 3 4 5 6 7 X (160) move about actively when s/he is exploring
new surroundings?

When your baby was approached by an unfamiliar person when you and s/he
were out (for example, shopping), how often did the baby:

1 2 3 4 5 6 7 X (161) show distress?

1 2 3 4 5 6 7 X (162) cry?

When an unfamiliar adult came to your home or apartment, how often did your
baby:

1 2 3 4 5 6 7 X (163) allow her/himself to be picked up without
protest?

1 2 3 4 5 6 7 X (164) cry when a visitor tried to pick her/him up?

When in a crowd of people, how often did the baby:

1 2 3 4 5 6 7 X (165) seem to enjoy him/herself?

Did the baby seem sad when:

1 2 3 4 5 6 7 X (166) caregiver is gone for an unusually long period
of time?

1 2 3 4 5 6 7 X (167) left alone/unattended in a crib or a playpen
for an extended period of time?

When you were busy with another activity, and your baby was not able to get your attention, how often did s/he:

1 2 3 4 5 6 7 X (168) become sad?

1 2 3 4 5 6 7 X (169) cry?

When your baby saw another baby crying, how often did s/he:

1 2 3 4 5 6 7 X (170) become tearful?

1 2 3 4 5 6 7 X (171) show distress?

When familiar relatives/friends came to visit, how often did your baby:

1 2 3 4 5 6 7 X (172) get excited?

1 2 3 4 5 6 7 X (173) seem indifferent?

Soothing Techniques

Have you tried any of the following soothing techniques in the last two weeks? If so, how quickly did your baby soothe using each of these techniques? Circle (X) if you did not try the technique during the LAST TWO WEEKS.

When rocking your baby, how often did s/he:

1 2 3 4 5 6 7 X (174) soothe immediately?

1 2 3 4 5 6 7 X (175) not soothe immediately, but in the first two
minutes?

1 2 3 4 5 6 7 X (176) take more than 10 minutes to soothe?

When singing or talking to your baby, how often did s/he:

1 2 3 4 5 6 7 X (177) soothe immediately?

1 2 3 4 5 6 7 X (178) not soothe immediately, but in the first two
minutes?

1 2 3 4 5 6 7 X (179) take more than 10 minutes to soothe?

When walking with the baby, how often did s/he:

1 2 3 4 5 6 7 X (180) soothe immediately?

1 2 3 4 5 6 7 X (181) not soothe immediately, but in the first two
minutes?

1 2 3 4 5 6 7 X (182) take more than 10 minutes to soothe?

When giving him/her a toy, how often did the baby:

1 2 3 4 5 6 7 X (183) soothe immediately?

1 2 3 4 5 6 7 X (184) not soothe immediately, but in the first two
minutes?

1 2 3 4 5 6 7 X (185) take more than 10 minutes to soothe?

When showing the baby something to look at, how often did s/he:

1 2 3 4 5 6 7 X (186) soothe immediately?

1 2 3 4 5 6 7 X (187) not soothe immediately, but in the first two
minutes?

1 2 3 4 5 6 7 X (188) take more than 10 minutes to soothe?

When patting or gently rubbing some part of the baby's body, how often did
s/he:

1 2 3 4 5 6 7 X (189) soothe immediately?

1 2 3 4 5 6 7 X (190) not soothe immediately, but in the first two
minutes?

1 2 3 4 5 6 7 X (191) take more than 10 minutes to soothe?

A.12 Baby Behaviour Diary



The FirstFeed Study

Study-ID _____

BABY BEHAVIOUR DIARY AT _____WEEKS

Contains:

Instructions for completing the diary

A sample diary, to show what a completed diary might look like.

Three-day diary for filling in

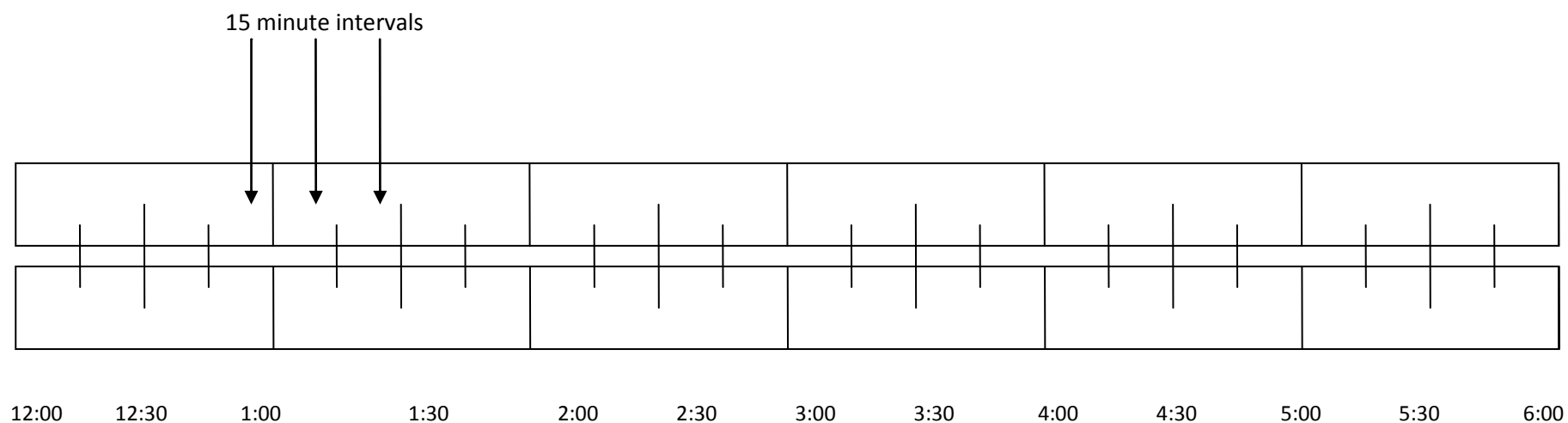
Please telephone the researcher if you have any questions about filling in the diary

(tel.: 0141-201-9341 or 07876 214 464)

INSTRUCTIONS FOR COMPLETING THE DIARY

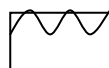
This diary is designed to enable you to record your baby's behaviour and your activities with your baby over a continuous 24-hour period. As you can see, the day is divided into four blocks of 6 hours each. For example:

The top time ruler is for recording your baby's behaviour



The bottom time ruler is for recording your activities or those of other care-givers

The record is filled in by shading on the 'time rulers' using the appropriate type of shading. An example is given here. Note that activities or behaviour don't have to last for 15 minutes to be filled in. The length of shading in tells us how long they lasted for. If you can be accurate to within about 5 minutes, that will be accurate enough.



Sleeping



Fussy: your baby is unsettled and irritable,
and may be vocalising but not continuously crying



Awake and content



Crying: periods of prolonged,
distressed vocalisation

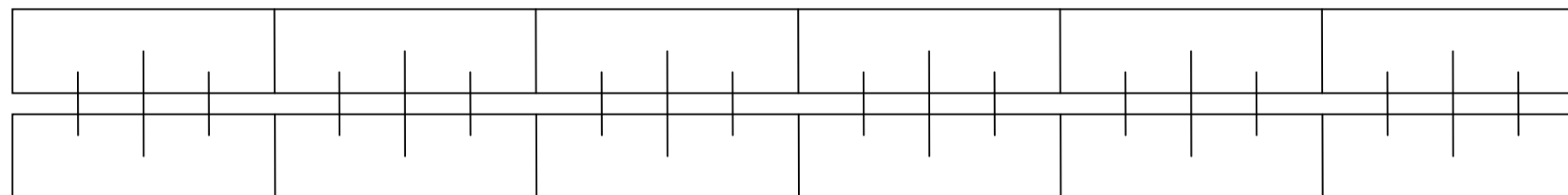


Awake and active



Feeding

The top time ruler is for recording your baby's behaviour



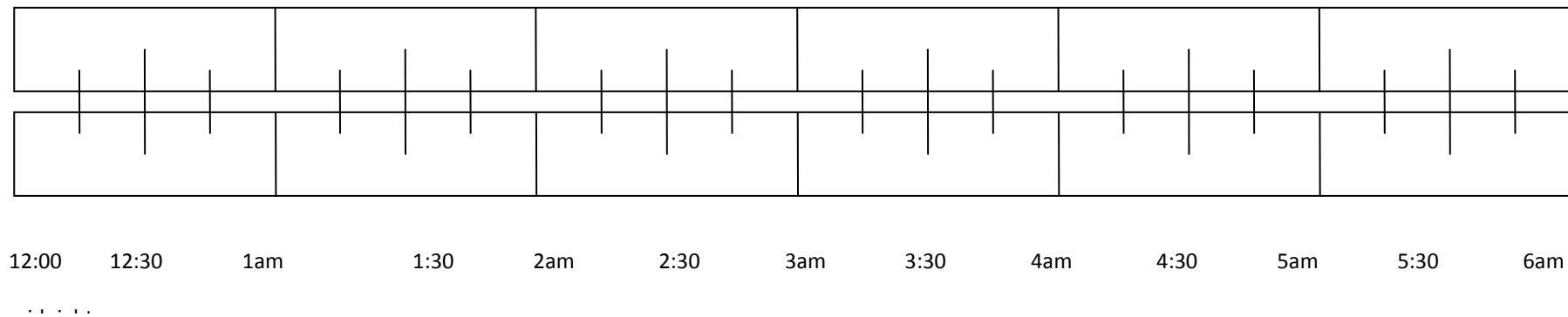
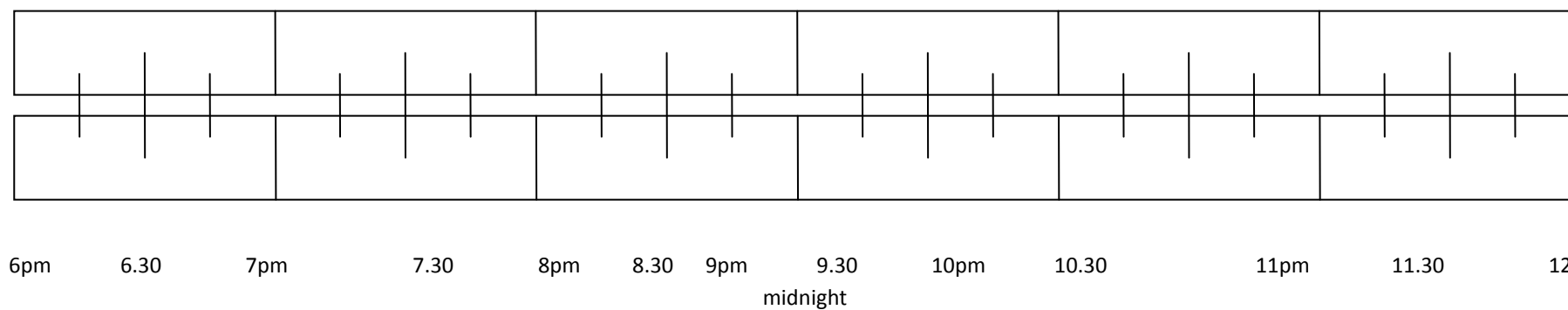
12:00 12:30 1:00 1:30 2:00 2:30 3:00 3:30 4:00 4:30 5:00 5:30 6:00

The bottom time ruler is for recording your activities or those of other care-givers: H = held or carried; C = bath or nappy change; P = play

Day 1. Date _____

6am 6.30 7am 7.30 8am 8.30 9am 9.30 10am 10.30 11am 11.30 12 noon

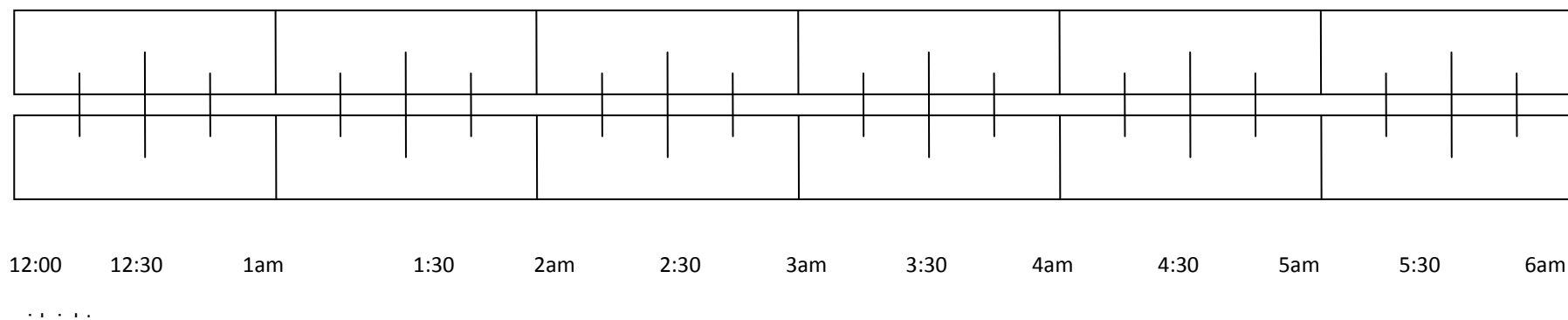
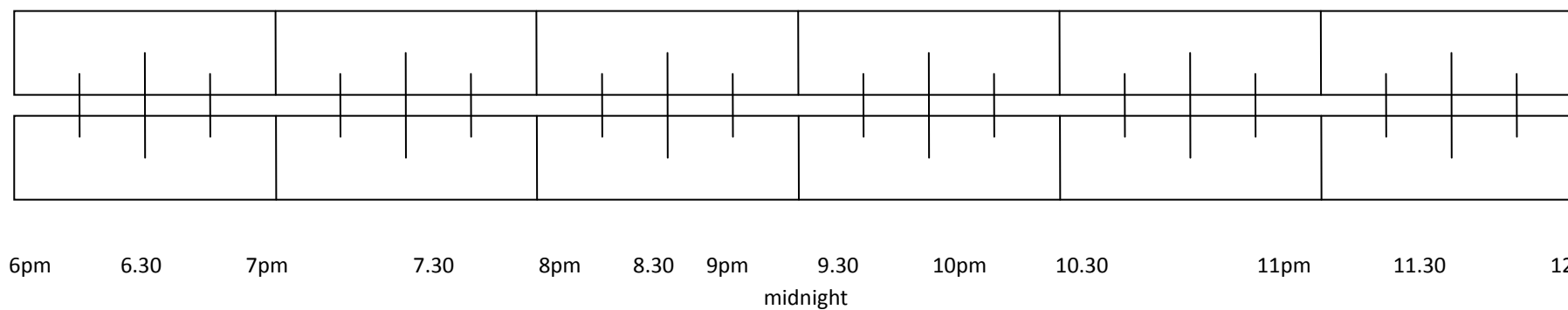
12:00 12:30 1pm 1:30 2pm 2:30 3pm 3:30 4pm 4:30 5pm 5:30 6pm



Day 2. Date _____

6am 6.30 7am 7.30 8am 8.30 9am 9.30 10am 10.30 11am 11.30 12 noon

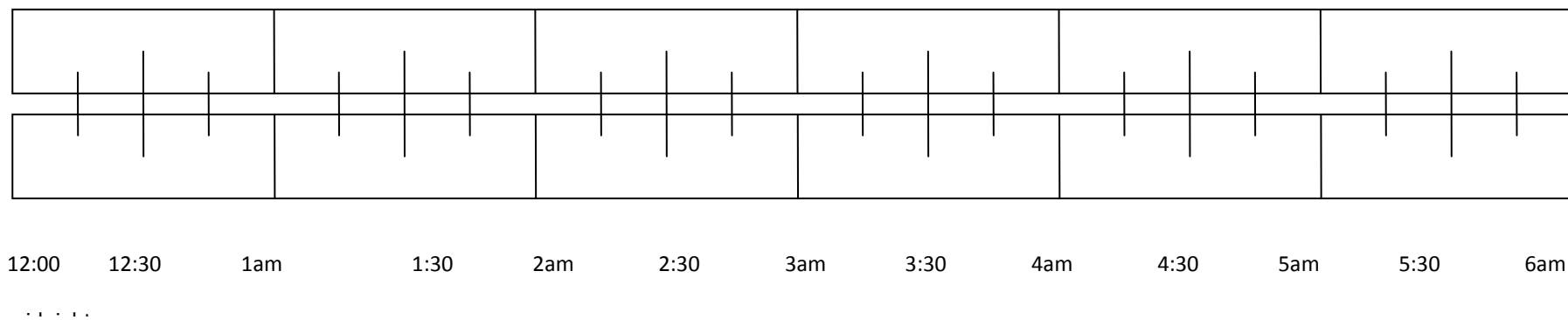
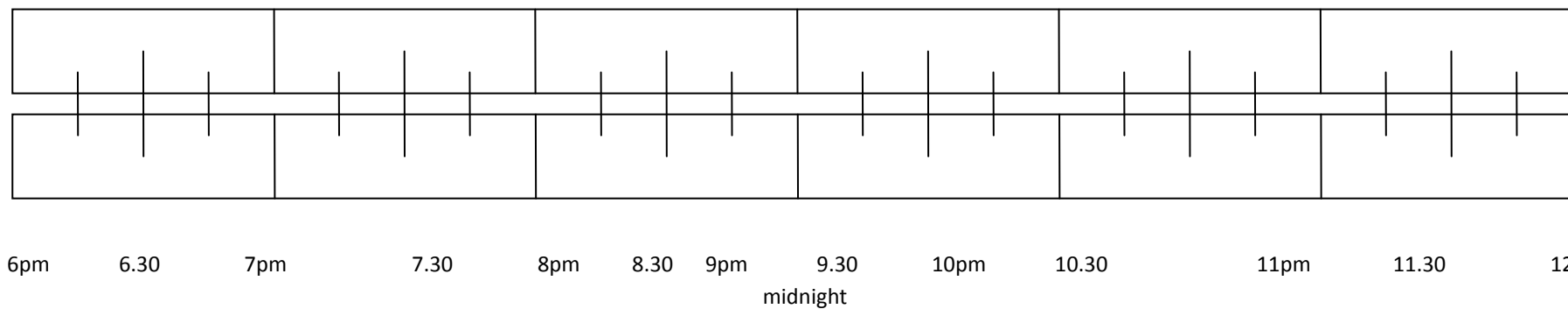
12:00 12:30 1pm 1:30 2pm 2:30 3pm 3:30 4pm 4:30 5pm 5:30 6pm



Day 3. Date _____

6am 6.30 7am 7.30 8am 8.30 9am 9.30 10am 10.30 11am 11.30 12 noon

12:00 12:30 1pm 1:30 2pm 2:30 3pm 3:30 4pm 4:30 5pm 5:30 6pm



A.13 Data Sheet 7: Readiness for complementary foods**Readiness for complementary feeding**

Can your baby hold his/her head up?

Yes...☐ No...☐

Can your baby sit up in a high chair?

Yes...☐ No...☐

Can your baby reach out and touch or grab things?

Yes...☐ No...☐

Does your baby put hand to mouth?

Yes...☐ No...☐

Does your baby explore toys by putting them in the mouth?

Yes...☐ No...☐

Is your baby still hungry after breast-feeding?

Yes...☐ No...☐

Comments:

A.14 Complementary feeding questionnaire



Introducing Complementary Foods

A. Breast-feeding.

1. Is your baby still breast-fed?

Yes...☐ No...☐ →Go to Q9

2. Does your baby usually feed from both breasts at each feeding?

Yes...☐ No...☐ Baby is only fed expressed milk...☐ →Go to Q5

3. Does your baby usually let go of the breast him or herself?

- ☐ Yes, both breasts
- ☐ Yes, first breast only
- ☐ Yes, second breast only
- ☐ No

4. About how long does an average breast-feeding last?

- ☐ Less than 10 minutes
- ☐ 10 to 19 minutes
- ☐ 20 to 29 minutes
- ☐ 30 to 39 minutes
- ☐ 40 to 49 minutes
- ☐ 50 or more minutes

5. In an average 24-hour period, what is the LONGEST time for you, the mother, between breast-feedings or expressing milk? Please count the time from the start of one breast-feeding or expressing session to the start of the next. Please think of time between feedings during both night and day to find the longest time. (WRITE IN THE NUMBER OF HOURS AND MINUTES)

_____ HOURS AND _____ MINUTES

6. How many times in the past 7 days was your baby fed expressed breast milk to drink? (Write 0 if your baby was not fed expressed milk)_____ TIMES,

IF 0 → Go to Q9

7. How often does your baby drink all of his or her cup or bottle of expressed milk?

Never...☐ Rarely...☐ Sometimes...☐ Most of the time...☐ Always...☐

8. How often is your baby encouraged to finish a cup or bottle if he or she stops drinking before the expressed breast milk is all gone?

Never...☐ Rarely...☐ Sometimes...☐ Most of the time...☐ Always...☐

B. Stopping Exclusive Breast-feeding

9. Have you completely stopped breast-feeding and expressing milk for your baby?

Yes...☐ No...☐ →Go to Q13

10. Did you breast-feed as long as you wanted to?

Yes...☐ No...☐

11. How old was your baby when you completely stopped breast-feeding and expressing milk? _____ WEEKS

12. How important was each of the following reasons for your decision to stop breast-feeding your baby? (PLEASE ANSWER EACH ITEM)

NOT AT ALL NOT VERY SOMEWHAT VERY

IMPORTANT IMPORTANT IMPORTANT IMPORTANT

My baby had trouble sucking or latching on...

☐ ☐ ☐ ☐

My baby became sick and could not breast-feed...

☐ ☐ ☐ ☐

My baby began to bite...

☐ ☐ ☐ ☐

My baby lost interest in nursing or began to wean him or herself...

☐ ☐ ☐ ☐

NOT AT ALL NOT VERY SOMEWHAT VERY

IMPORTANT IMPORTANT IMPORTANT IMPORTANT

My baby was old enough that the difference between breast milk and formula no longer mattered...

☐ ☐ ☐ ☐

Breast milk alone did not satisfy my baby...

☐ ☐ ☐ ☐

I thought that my baby was not gaining enough weight...

☐ ☐ ☐ ☐

A health professional said my baby was not gaining enough weight...

☐ ☐ ☐ ☐

I had trouble getting the milk flow to start...

☐ ☐ ☐ ☐

I didn't have enough milk...

☐ ☐ ☐ ☐

My nipples were sore, cracked, or bleeding...

☐ ☐ ☐ ☐

My breasts were overfull or engorged...

☐ ☐ ☐ ☐

My breasts were infected or abscessed...

☐ ☐ ☐ ☐

My breasts leaked too much...

☐ ☐ ☐ ☐

Breast-feeding was too painful...

☐ ☐ ☐ ☐

Breast-feeding was too tiring...

☐ ☐ ☐ ☐

NOT AT ALL NOT VERY SOMEWHAT VERY

IMPORTANT IMPORTANT IMPORTANT IMPORTANT

I was sick or had to take medicine...

☐ ☐ ☐ ☐

Breast-feeding was too inconvenient...

☐ ☐ ☐ ☐

I did not like breast-feeding...

☐ ☐ ☐ ☐

I wanted to be able to leave my baby for several hours at a time...

☐ ☐ ☐ ☐

I wanted to go on a weight loss diet...

☐ ☐ ☐ ☐

12. How important was each of the following reasons for your decision to stop breast-feeding your baby? (PLEASE ANSWER EACH ITEM)

NOT AT ALL NOT VERY SOMEWHAT VERY

IMPORTANT IMPORTANT IMPORTANT IMPORTANT

I wanted to go back to my usual diet...

☐ ☐ ☐ ☐

I wanted to smoke again or more than I did while breast-feeding...

☐ ☐ ☐ ☐

I had too many household duties...

☐ ☐ ☐ ☐

I could not or did not want to express or breast-feed at work...

☐ ☐ ☐ ☐

Expressing milk no longer seemed worth the effort that it required...

☐ ☐ ☐ ☐

I was not present to feed my baby for reasons other than work...

☐ ☐ ☐ ☐

NOT AT ALL NOT VERY SOMEWHAT VERY

IMPORTANT IMPORTANT IMPORTANT IMPORTANT

I wanted or needed someone else to feed my baby...

☐ ☐ ☐ ☐

Someone else wanted to feed the baby...

☐ ☐ ☐ ☐

I did not want to breast-feed in public...

☐ ☐ ☐ ☐

I wanted my body back to myself...

☐ ☐ ☐ ☐

I became pregnant or wanted to become pregnant again...

☐ ☐ ☐ ☐

C. Introducing infant formula

13. Was your baby fed infant formula in the past 7 days, by you or by anyone else?

Yes...☐ No...☐ →Go to Q18

14. In the past 7 days, about how many ounces of formula did your baby drink at each feeding? 1 - 2...☐ 3 - 4...☐ 5 - 6...☐ 7 - 8...☐ >8...☐

1 ounce = 30 ml

15. How often does your baby drink all of his or her bottle of formula?

Never..... ☐ Rarely..... ☐ Sometimes..... ☐ Most of the time.....
☐ Always..... ☐

16. How often is your baby encouraged to finish a bottle if he or she stops drinking before the formula is all gone?

Never...☐ Rarely...☐ Sometimes...☐ Most of the time...☐ Always...☐

17. What type of infant formula was your baby fed in the last 7 days?

(PLEASE "X" ALL THAT APPLIES)

- ☐ Ready to feed
- ☐ Powder from can that makes more than one bottle
- ☐ Liquid concentrate
- ☐ Powder from single serving packs

D. Introducing complementary foods

18. How important was each of the following reasons for feeding your baby solid food for the very first time? Solid foods are foods such as cereal, baby foods, or table food.

NOT AT ALL NOT VERY SOMEWHAT VERY

IMPORTANT IMPORTANT IMPORTANT IMPORTANT

My baby was breast-feeding too much...

☐ ☐ ☐ ☐

My baby seemed hungry a lot of the time...

☐ ☐ ☐ ☐

I didn't have enough milk...

☐ ☐ ☐ ☐

My baby was not gaining enough weight...

☐ ☐ ☐ ☐

I wanted to feed my baby something in addition to breast milk...

☐ ☐ ☐ ☐

It would help my baby sleep longer at night...

☐ ☐ ☐ ☐

My baby was old enough to begin eating solid food...

☐ ☐ ☐ ☐

My baby had a medical condition that might be helped by feeding solid food...

☐ ☐ ☐ ☐

NOT AT ALL NOT VERY SOMEWHAT VERY

IMPORTANT IMPORTANT IMPORTANT IMPORTANT

A doctor or other health professional said my baby should begin eating solid foods...

☐ ☐ ☐ ☐

Friends or relatives said my baby should begin eating solid foods...

☐ ☐ ☐ ☐

My baby wanted food I ate or in other ways showed an interest in solid food...

☐ ☐ ☐ ☐

19. About how often did you introduce new foods (such as a specific type of cereal, fruit, vegetable, or meat) to your baby over the past 2 weeks?

- ☐ No new foods in the past 2 weeks
- ☐ About 1 new food every 2 days
- ☐ About 1 new food per week or less often
- ☐ About 1 new food every day
- ☐ About 1 new food every 4 or 5 days
- ☐ More than 1 new food every day
- ☐ About 1 new food every 3 days

Date you completed this form: Day _____ Month _____ Year _____

Appendix B: The doubly-labelled water method: Principles, calculations and assumptions

B.1 Isotopic abundance and enrichment

B.2 Dilution spaces

B.3 Total body water and body composition

B.4 Isotope elimination rates

B.5 CO₂ production rate and total energy expenditure

B.6 Energy cost of growth

B.7 Metabolisable energy intake

B.8 Breast milk intake

B.9 Metabolisable energy content of breast milk

B.10 Seven assumptions regarding the behaviour of isotopes

Section 2.3 described the use of the DLW method for the present study. The present appendix is a description of the principles of the DLW technique with focus on the equations needed to derive the outcome variables as well as the assumptions behind those equations. As part of this description there will be references to sections 1.2.1, 2.3.5 and 2.3.6.

In general, there are seven underlying assumptions about the behaviour of isotopes, which affect the equations below. In addition, there are a number of estimates for constants included in the equations, and these will be dealt with together with the concerned equation. These estimates are often averaged values, which are used where biological variation is considered to be random and tend to equal out.

B.1 Isotopic abundance and enrichment

The definition of an isotope was described in section 1.2.1, where it was briefly stated that the heavy isotopes of hydrogen, deuterium, and oxygen, oxygen-18 are present in abundance levels all around us. Thus the term *isotopic abundance* merely reflects the presence of different isotopes of a molecule. Measuring isotopic abundance is counting atoms of different molecular weights. The result of measuring isotopic abundance in terms of number of atoms of any particular weight, depend on how many atoms are counted, i.e. how big the sample is. Therefore it is more convenient to standardise the expression of isotopic abundance as a ratio of heavy to light isotopes. Hence when referring to abundance of deuterium the ratio is $^2\text{H}/^1\text{H}$ in a sample of water, and for oxygen-18 the ratio is $^{18}\text{O}/^{16}\text{O}$. Expressing isotopic abundance as a ratio can be compared to the expression of a concentration. If a sample contains more heavy to light isotopes than the natural abundance level (see below), it is said to be *enriched*.

The isotopic enrichment of a sample from a participant is not only influenced by the dose of isotope given, but also by the initial baseline level, the natural abundance level of the isotope. Knowing the baseline level is very important for later calculations. Since natural abundance levels vary with geographical location, diet, and season, it is convenient to standardise all isotopic measurement relative to a reference. For this purpose the International Atomic Energy Agency (IAEA) has defined a reference water as the Vienna - Standard Mean Ocean Water (SMOW). This reference water can be bought from the IAEA and each laboratory analysing isotope ratios then make up their own laboratory reference expressed relative to SMOW. The laboratory reference is then applied during every single run of analysis on the mass spectrometer, to be able to correct the data for any drift during analysis (corrected by a factor of known value/observed value of reference water). Drift might be caused by changes in temperature, humidity or “temperament” of the mass spectrometer. Data obtained from the mass spectrometer is expressed as a ratio of $^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$, respectively, and denoted delta, δ (unit: ‰).

B.2 Dilution spaces

Initial dilution spaces

The dilution space (also called the pool size, when measured in moles; $\times 18.02$ g/mol) is the amount of body water which the isotopes equilibrate in after dose administration (section 2.3.5). The dilution spaces of the isotopes were estimated from back-extrapolation of the elimination curves to the intercept of the Y-axis (time 0), when the dose was administered. The equation used for determining dilution spaces for deuterium (N_D) and oxygen-18 (N_O) at t_0 ²⁵⁰ is described in section 2.3.5.

Assumption for dilution spaces

Determining dilution space in this way, assumes that the dose of DLW equilibrates instantaneously. In practice, it takes 3 - 4 hours for the DLW to equilibrate, and during this time, a little bit of the isotope will already have been eliminated through water efflux and CO₂ exhalation³²⁹. Therefore the dilution spaces determined this are strictly speaking only theoretical values. However, most of the isotope that is lost, is in equilibrium and not important. Therefore, the error introduced by this assumption is usually 1% or less³²⁹. The dilution spaces were used to calculate the space ratio (see below) and TBW, as described in section 2.3.5. The dilution spaces used in later calculations had to be corrected for changes in the water pool during the week of the measurement due to growth. This was done by correcting with a factor, gamma, which was the natural logarithm of the average of the weights measured at the beginning and at the end of each measurement.

Dilution space ratio

The ratio of dilution spaces for the isotopes deuterium/oxygen-18 is termed the space ratio. Since deuterium is more prone to exchange with non-aqueous molecules than oxygen-18, the space ratio is never <1 . The space ratio varies as a result of biological variation, but is mainly affected by analytical error. It would not be possible to correct for any analytical error, by assuming that either of the dilution spaces would be more correct. Neither would it be possible to correct for analytical error, by using a mean value, since this value would also represent a mean of the analytical error.

Therefore, a Bayesian approach was used instead, where the more extreme the space ratio was, the more it was corrected towards the mean value of 1.034. After this correction, a range of 1.000 - 1.090 was used as a cut-off on the elimination spreadsheets for acceptable analytical error (section 2.3.5).

B.3 Total body water and body composition

Total body water

If the isotopes equilibrated only in the body water pool, as in a single compartment system, then TBW would be equal to the dilution space calculated from either oxygen-18 or deuterium. However, some of the isotope exchange with oxygen and hydrogen atoms in other molecules (non-aqueous exchange), and this happens to a greater extent (and with greater variability) for deuterium than for oxygen-18²⁵⁰. Therefore, TBW was calculated as described in section 2.3.5, correcting with a factor of 1.01 to correct for non-aqueous exchange of oxygen-18, and a factor of 1.04 to correct for non-aqueous exchange of deuterium³²⁹, and using an average of the two values for TBW.

Body composition

Body composition was divided into Fat Free Mass (FFM) and Fat Mass (FM) according to the two-compartment model, where FFM was defined as all of the body mass that is not fat. Of the two compartments, FFM is the most complex in its composition. FFM includes all of TBW, but the hydration coefficient of FFM varies with sex and age. Therefore, FFM was calculated proportionally to TBW, using sex- and age-specific hydration coefficients, as described in section 2.3.6 and outlined in table 2.2, which were derived from regression equations on data on the reference child²⁴⁹ (Section B.6). The variables of body composition were used to estimate E_{growth} as described in section 2.3.6.

B.4 Isotope elimination rates

The two-point and multi-point models

In general, there are two models for calculating isotope elimination rates. Both are based on the original model developed in the Lifson and colleagues²⁵¹ in the 1950's based on rodent studies.

In brief, the two-point method, proposed by Schoeller⁴⁸⁸, calculates isotope elimination rates from a start- and end-point strategy, where the initial enrichment is measured by sampling after a 3-4 hours equilibrium period. The multi-point method, described by Lucas and colleagues²⁷⁹, uses either regressions to derive isotope elimination rates from a series of time-points and estimates the initial enrichment by back extrapolation. The two-point method therefore provides one average value of elimination rates from the measurement period, whereas the multi-point method provides an average value for daily elimination. This average value can be further assessed with a standard error of the estimate.

Both methods have advantages and disadvantages. The advantages of the two-point method are fewer samples and therefore less sample analysis, and being more robust to changes in water turnover and CO₂ production. These changes are changes in rates of water influx as well water and CO₂ efflux. The disadvantages are that it is less robust to random errors in isotope enrichment and therefore has lower precision, and it assumes a fixed ratio of 1.03 between dilution spaces, which might result in errors, if there are any physiological variations in this ratio. In addition, the two-point method ideally requires a period of both pre-dose and post-dose fasting, to obtain the most accurate measurement of dilution spaces. In infants, this strategy would not be feasible due to their often irregular feeding routine, nor would it be ethical to require a healthy infant to be fasting for any length of time in an observational study.

The multi-point method has a higher precision, because it is more robust to random errors, and it does not need to assume a fixed dilution space ratio. Additional residual analysis provides extra information on error propagation, and the covariance between isotope residuals provides an assessment of analytical accuracy. The disadvantage is a higher burden on the participants in terms of sampling, and it is less robust to large changes in water turnover and CO₂ production^{305,325}. In general, errors have been found to be higher in the two-point method than the multi-point method, when used on infants²⁵⁰, and when accuracy and precision are both evaluated, the multi-point method comes out as superior³³¹. Moreover, if the study design incorporated a multi-point method, this could be converted to a two-point method in individual participants, if for instance the sampling had been inadequate. Therefore, the multi-point method was chosen for the First-Feed study.

The multi-point method elimination rates

The two regression lines of log-transformed values of enrichment of deuterium and oxygen-18 were produced in the elimination spreadsheet, as described in section 2.3.5 and shown in Figure 2.4. The least squares regression line fitted could be a log fit, a Poisson fit or an exponential fit. Elimination rates for deuterium (k_D) and oxygen-18 (k_O) were calculated as the regression coefficients of the log-transformed enrichment against time curves, which as the slopes of the curves in Figure 2.4.

B.5 CO₂ production rate and total energy expenditure

Once dilution spaces (N_O and N_D) and elimination rates (k_O and k_D) had been determined, V_{CO_2} could be calculated as the difference between the products of dilution space and elimination rate for each isotope, corrected for fractionation⁴⁸⁹, as described in section 2.3.5. From the calculated V_{CO_2} , TEE was then calculated using the Weir's equation²⁴³ and an estimated value of RQ of 0.85, as described in section 1.2.1.

Ideally, to calculate TEE, it would be desirable to use a value for the RQ (total CO₂ produced/O₂ consumed for the participant) representing the substrate oxidation that have taken place during the measurement period. However, this value is prone to variation due to variations in substrate oxidation and is very difficult to estimate precisely. Therefore an approximation in the form of a food quotient (CO₂ produced/O₂ consumed during oxidation of food) is used instead. For participants in nutrient balance, FQ should be of same value as RQ, but for infants that is not the case. An inaccuracy is introduced, since infants store some of the macronutrients consumed. However, after 4 months of age, the effect of growth on FQ is considered to be negligible³³⁴. An analysis conducted by Professor Wells showed only a small effect on the calculated TEE from using different values of RQ⁴²⁰. Therefore a conservative approach was taken of choosing the same FQ of 0.85 as published by others²⁷⁹ to get comparable data.

B.6 Energy cost of growth

The body weights measured on the first and last days of each measurement were used to estimate E_{growth} . This estimate assumed that the body composition stayed constant during the week of measurement.

Changes in FFM and FM were then used to calculate the accumulation of fat and protein, assuming that the energy deposited as carbohydrate is negligible, as described in section 2.3.6.

Corrections for age- and sex- specific values for the hydration coefficient was done based on regressions derived from data on the reference child, published by Fomon and colleagues²⁴⁹, based on the approach developed by Wells and Davies^{244,255} (Table 2.2). Monthly reference values of protein as percentage of FFM (protein%) and TBW as percentage of FFM (TBW%) were entered into SPSS® for each sex. Age was then converted from months to days, and Age² computed as “age*age”. Reference values for protein as a fraction of FFM and TBW as a fraction of FFM for boys and girls were regressed against age and age² to produce following regression equations:

Boys:

$$\% \text{protein in FFM} = 14.967 + 0.007 (\text{age}) - 8.2 \times 10^{-6} (\text{age}^2)$$

$$\% \text{TBW in FFM} = 80.642 - 0.007 (\text{age}) + 7.06 \times 10^{-6} (\text{age}^2)$$

Girls:

$$\% \text{protein in FFM} = 14.996 + 0.009 (\text{age}) - 1.0 \times 10^{-5} (\text{age}^2)$$

$$\% \text{TBW in FFM} = 80.671 - 0.009 (\text{age}) + 1.11 \times 10^{-5} (\text{age}^2)$$

Age- and sex- specific hydration coefficients were subsequently calculated as²⁵⁵:

$$\text{Boys: } 80.642 - 0.007 * \text{Age} + 0.00000706 * \text{Age} * \text{Age}$$

$$\text{Girls: } 80.671 - 0.009 * \text{Age} + 0.0000111 * \text{Age} * \text{Age}$$

FFM was then calculated as:

$$\text{FFM} = \text{TBW} / \text{age-sex-specific TBW fraction of FFM}$$

The age- and sex-specific protein as a fraction of FFM was calculated as:

$$\text{Boys: } 14.967 + 0.007 * \text{Age} - 0.0000082 * \text{Age} * \text{Age}$$

$$\text{Girls: } 14.996 + 0.009 * \text{Age} - 0.00001 * \text{Age} * \text{Age}$$

Protein mass in FFM was calculated as:

$$\text{Protein_mass} = \text{FFM} * \text{age-sex-fraction-protein in FFM}$$

This was done for values at day 1 and day 7 of each week of measurement. Finally, a correction was made for TBW as a fraction of body weight based on the regression (also for both day1 and day 7 of each measurement):

$$\text{Boys: } 0.730 - 0.002 * \text{Age} + 5.67 \times 10^{-6} * \text{Age}^2$$

$$\text{Girls: } 0.716 - 0.002 * \text{Age} + 4.97 \times 10^{-6} * \text{age}^2$$

Then a regression proportion was calculated as:

Age-TBW-fraction in weight (day 7)/Age-%TBW in weight (day 1),

which was used to correct the new TBW at day 7.

B.7 Metabolisable energy intake

The metabolisable energy intake was calculated as the sum of TEE and E_{growth} (based on the first law of thermodynamics, which claims that energy can neither be created nor destroyed, but can only be transformed between different forms). Therefore TEI equals the sum of TEE and E_{growth} . Furthermore, TEI is assumed to be equal to metabolisable energy requirements when the infants are growing normally (under optimal conditions).

B.8 Breast milk intake

Summarising water influx

To calculate breast milk intake, it was first necessary to calculate total water influx (W_{in}), summarised as water efflux (W_{eff}) plus water stored (W_{st}) during the measurement period. Water efflux was calculated as the dilution space and elimination rate of deuterium, corrected for fractionation, corrected for an environmental water influx estimated to be 6.3%²⁴⁴. Water stored was obtained from the measurements of weight gain, composition of this weight gain and water content of FFM:

$$W_{\text{in}} (\text{g}) = W_{\text{eff}} + W_{\text{st}}$$

$$\text{where } W_{\text{eff}} (\text{g}) = N_D k_D / f_2 \times 106.3$$

$$\text{and } W_{\text{st}} (\text{g}) = \Delta \text{BW} \times \% \text{FFM} \times \text{hydration coefficient}$$

The calculated total water influx comprised of milk intake (milk W_{in}), any non-milk water intake (non-milk W_{in}), and environmental water influx (EWI). EWI is primarily the water exchange between atmospheric and alveolar water, and has

been estimated in 16 8-20 month-old infants by Fjeld and colleagues to be 7% of the total water influx²⁴⁵, and by Wells & Davies it has been found to be 6.3% in 21 12-week-old infants²⁴⁴. Non-milk water intake was assumed to be negligible for the infants that were EBF. For other infants, non-milk water was estimated weighed records of all food intake during the measurement. Finally, a correction was made for the water influx from oxidation of macronutrients in the breast milk. Coward found that metabolic oxidation of macronutrients in breast milk provided 0.11 g water per g breast milk, while the water content of breast milk was 0.85 g water per g breast milk⁴¹⁸:

$$\text{Total } W_{\text{in}} = \text{milk } W_{\text{in}} + \text{non-milk } W_{\text{in}} + \text{EWI},$$

$$\text{EWI} = 6.3\% \text{ of Total } W_{\text{in}} \Rightarrow$$

$$\text{Oral } W_{\text{in}} = 0.937 \times \text{Total } W_{\text{in}}$$

$$\text{Oral } W_{\text{in}} = \text{milk } W_{\text{in}} + \text{non-milk } W_{\text{in}} \Rightarrow$$

$$\text{milk } W_{\text{in}} = \text{Oral } W_{\text{in}} - \text{non-milk } W_{\text{in}}$$

$$\text{Milk intake} = \text{milk } W_{\text{in}} / 0.96$$

B.9 Metabolisable energy content of breast milk

The average daily metabolisable energy content of breast milk (kJ/g) was determined from the metabolisable energy intake (kJ) divided by the metabolisable milk intake (g).

B.10 Seven assumptions regarding the behaviour of isotopes

For the equations used to derive the outcome variables, a number of assumptions had to be made, which do not hold true for a biological system. In order to fully appreciate the method of DLW, it was important to understand to which extent the assumptions held true and to which extent they affected accuracy, precision or both. Following assumptions about the behaviour isotopes are stated as part of the principle of the DLW method, cited from reports by the International Atomic Energy Agency^{305,325}.

Wells has summarised how they are analysed for their impact on the accuracy and precision of the DLW method⁴²⁰:

- 1) The volume of the body water pool remains constant throughout the measurement period.
- 2) The rates of water influx, and water and CO₂ efflux are constant throughout the measurement period.
- 3) The isotopes label only the H₂O and CO₂ in the body (no isotopic exchange).
- 4) The isotopes leave the body only in the forms of H₂O and CO₂.
- 5) The concentrations of isotopes in H₂O and CO₂ leaving the body are the same as those in body water at that time (no fractionation).
- 6) No H₂O or CO₂ that has left the body re-enters the body.
- 7) The natural abundance or “background” levels of the isotopes remain constant during the measurement interval.

Assumption 1

The TBW is assumed to be constant during measurement. This is probably not the case for an infant, for instance due to growth. Therefore a correction for change in size of TBW during the sampling period had to be made. This was done by measuring growth velocity during the measurement period (increase in weight in g), and add a correction factor, gamma, which was the natural logarithm of the average of the body weights measured before dosing and at the end of the measurement week. Using this approach, the assumption is less likely to introduce any large error to the DLW method⁴²⁰.

Assumption 2

Water turnover and V_{CO₂} is assumed to be constant during the measurement. If these are not constant throughout the measurement, this will affect the elimination rates, k_D and k_O. When using the multi-point model, the effects on the calculated elimination rates depend on which regression fit is used. A linear regression fit is more robust, than an exponential fit, if the variation in CO₂ production occurs at the beginning of the period, since these data-points have more influence and the final elimination curve due to the higher enrichments at the beginning³²⁵. In general, the calculation of V_{CO₂} is less sensitive to changes in water turnover than to changes in CO₂ exhalation, since water turnover is represented in the elimination of both isotopes, and therefore tends to equal out. The two-point method is more robust to this kind of variation.

Assumption 3

The isotopes are assumed to only equilibrate in the water and the CO₂ of the body. As explained in section B.3, if this was true, the dilution spaces for deuterium and oxygen-18 would have the same value and would be equal to TBW. However, in practice the isotopes exchange with non-aqueous oxygen and hydrogen elsewhere in the body. Deuterium can exchange with hydrogen in both carbohydrates, proteins and fat molecules, and the latter is particularly the case in growing infants who accumulate fat during the measurement. Therefore some deuterium is lost from the water pool, when being incorporated into fat acids. Oxygen-18 can exchange with oxygen mainly in proteins, but this happens to a lesser extent than the deuterium/hydrogen exchange. Therefore the dilution spaces for deuterium and oxygen-18 are not of the same value, and they both overestimate TBW, because the ratios of isotopes are measured as lower, than if there were no exchange. Studies suggest that using oxygen-18 as a value for TBW will overestimate TBW by about 2% (in piglets)³³⁰, while the overestimation of TBW from the dilution space of deuterium is in the order of 3-4% but varies from 1 - 15%⁴⁹⁰.

In infants this degree of overestimation of TBW from deuterium might be more prone to inaccuracy due to variation in fat mass and fat accretion. Therefore, TBW was calculated as an average based on the dilution spaces of both oxygen-18 and deuterium. In theory, this assumption also affects the calculation of elimination rates. However, testing this on data from a study of young adults suggested, that the effects on the rate constant tended to cancel out and produce errors of less than 1%⁴¹⁷.

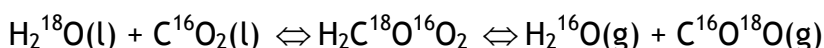
Assumption 4

The isotopes should only leave the body only through water and CO₂ efflux. In theory, the isotopes could also leave the body in the other compounds of excretions and secretions. However, this has been estimated to be less than 0.5% of total isotope elimination and is therefore deemed negligible⁴¹⁶.

Assumption 5

Fractionation occurs from chemical reactions, where the isotopic abundances are not the same in reactants and products, even though reactants and products themselves are in equilibrium.

Fractionation affects the equilibrium reaction catalysed by the carbonic anhydrase:



The products in the water efflux will then not have the same isotopic enrichment as in the body, and the heavy isotopes will be eliminated from the body in a rate that is different from the lighter isotopes. Therefore, when fractionation takes place, the turnover of DLW does not represent the turnover of water, or the production of CO_2 . Hence it is necessary to correct for this fractionation, and fractionation factors are calculated as the ratio of heavy to light isotope in the product divided by the same of the reactant. From the equation above, the relevant fractionation factors are:

- 1) Fractionation of $^2\text{H}_2\text{O}$ between liquid and gas (f_1)
- 2) Fractionation of H_2^{18}O between liquid and gas (f_2)
- 3) Fractionation of exchanged $\text{H}_2^{18}\text{O} \leftrightarrow \text{C}^{16}\text{O}_2$ (f_3)

The fractionation factors were incorporated in the equation for calculation of V_{CO_2} , as described in section 2.3.5.

Assumption 6

The risk of isotope re-entering the body is considered to be practically none, since it would only be relevant if the infants were drinking isotope enriched liquids, but this should not be the case when they are exclusively breast-fed and hence only drinking breast milk.

Assumption 7

Establishing elimination rates of the isotopes using the multi-point method, assumes that the natural abundance level of the isotopes remain constant throughout the elimination period. The effect of this assumption not holding true concerns both an effect of changes in the absolute background levels of the isotopes, as well as a change in the ratio between deuterium and oxygen-18.

But it is probably a fair assumption for exclusively breast-fed infants, and where the families did not travel during the study. However, some of the infants had been introduced to solids by the 2nd time point and it has been shown that weaning affects the accuracy of the DLW method, if 25% or more of breast-milk is substituted with formula³¹⁸.

Appendix C: Author bibliography

C.1 List of published papers arising from the First-Feed study

Papers published from this thesis have been attached (Appendix C.4).

- I. Nielsen SB, Wells JCK, Slater C, Fewtrell MS, Reilly JJ. Administering labelled water to exclusively breast-fed infants in studies involving stable isotope dilution techniques. *Isotopes in Environmental and Health Studies* 2011;47(1):18-25.
- II. Nielsen SB, Reilly JJ, Fewtrell MS, Eaton S, Grinham J, Wells JCK. Adequacy of milk intake during exclusive breastfeeding: A longitudinal study. *Pediatrics* 2011;128(4): e907-e914.
- III. Nielsen SB, Wells JCK, Fewtrell MS, Eaton S, Grinham J, Reilly JJ. Validation of energy requirement references for exclusively breast-fed infants. *British Journal of Nutrition* 2012 (In Press)
- IV. Nielsen SB. Can mothers manage exclusive breastfeeding for 6 months? *Pediatrics for Parents* 2012 (In Press).

C.2 List of published abstracts arising from the First-Feed study

- Nielsen SB, Wells JCK, Fewtrell MS, Reilly JJ. Feeding behaviour and energy balance in 4- to 6-month old infants: adaptive changes during exclusive breast-feeding. *Proceedings of the Nutrition Society* 2008; 67(OCE4): E220.
- Nielsen SB, Reilly JJ, Fewtrell MS, Wells JCK. Adaptations to prolonged exclusive breastfeeding: Longitudinal study of mother-infant pairs following the World Health Organization recommendation on infant feeding. *Journal of Human Lactation* 2010; 26(4): 428.
- Nielsen SB, Reilly JJ, Fewtrell MS, Wells JCK. Adaptations to prolonged exclusive breastfeeding: Longitudinal study of mother-infant pairs following WHO recommendation on infant feeding. *Breastfeeding Medicine* 2010; 5(6): 327.

C.3 List of other achievements during this Ph.D.

Poster presentations

11-2006: Yorkhill Research Prize Evening: Nielsen SB, Michaelsen KF. The effect of milk type on milk intake and energy distribution in healthy infants aged 9 to 12 months.

01-2008: ISHRML 14th International Conference: Nielsen SB, Wells JCK, Fewtrell MS, Reilly JJ. Infant energy balance and growth during exclusive breast-feeding for six months.

03-2008: Annual Meeting of the Scottish Section of the Nutrition Society. Nielsen SB, Wells JCK, Fewtrell MS, Reilly JJ. Feeding behaviour and energy balance in 4- to 6-month old infants: adaptive changes during exclusive breast-feeding.

06-2009: Annual Meeting of ISBNPA. Nielsen SB, Wells JCK, Fewtrell MS, Reilly JJ. Adaptive changes in feeding behaviour and infant energy balance during exclusive breast-feeding. Preliminary results.

03-2012: Nutrition and Growth. Nielsen SB, Wells JCK, Fewtrell MS, Reilly JJ. Growth in a sample of UK infants exclusively breast-fed according to WHO recommendation.

Oral presentations

11-2006: Yorkhill Academic Seminar. Diet and intakes of infants during weaning.

02-2008: Yorkhill Academic Seminar. The First-Feed Study: For infant research studies: Breast-feeding Exclusively for an extended duration.

04-2008: Academic Day of the Scottish Federation of University Women. The First-Feed study: Background, methodology and recruitment.

11-2008: Yorkhill Research Day. Milk intake in exclusively breast-fed infants. Preliminary results.

06-2009: ESPGHAN summer school. Milk intake and energy expenditure in exclusively breast-fed infants from 4- to 6-months. Preliminary results.

10-2009: Yorkhill Academic Seminar. Changes in lactation performance and infant energy balance in exclusively breast-fed infants from 4- to 6-months of age.

04-2010: Inverclyde Breast-feeding Mums Annual Meeting. Milk intake and growth in exclusively breast-fed infants from 3½- to 6 months of age.

06-2010: Scottish Breast-feeding Conference. Title as before.

09-2010: Scottish Federation of University Women. Title as before.

10-2010: 15th International Conference of ISHRML. Adaptations to prolonged exclusive breastfeeding: Longitudinal study of mother-infant pairs following WHO recommendation on infant feeding.

10-2010: 15th Annual Meeting of the Academy of Breast-feeding Medicine. Adaptations to prolonged exclusive breastfeeding: Longitudinal study of mother-infant pairs following WHO recommendation on infant feeding.

Prizes

1st prize of long presentations at the Yorkhill Research Day 2008.

1st prize of platform presentations at the 15th International Meeting of the Academy of Breastfeeding Medicine, October 2010.

Grants

2006 - 2008: Part-time student grant, Blegdalens Erhvervs- og Uddannelsesfond (Eng.: Blegdalen's Business and Educational Fund): £18,000.

2008 - 2010: Full-time student grant, Yorkhill Children's Foundation: £32,000.

2008 - 2010: Part Scholarship, University of Glasgow, Medical Faculty Graduate School: £6,600.

02-2008: Travel grant, Australian Government: (AUS)\$1750.

03-2008: Travel grant, Roberts' Travelling Fellowship: £562.

05-2009: Travel grant, Roberts' Travelling Fellowship: £750.

05-2009: Travel grant, Yorkhill Children's Foundation/Lawrence Weaver: £500.

10-2010: Travel grant, ISRHML: (US)\$1000.

10-2010: Travel grant, Yorkhill Children's Foundation: £978.

Exam

August 2010: CAPM - Certificate of Associate Project Manager (Project Management Institute accredited).

C.4 Papers published from this Ph.D.

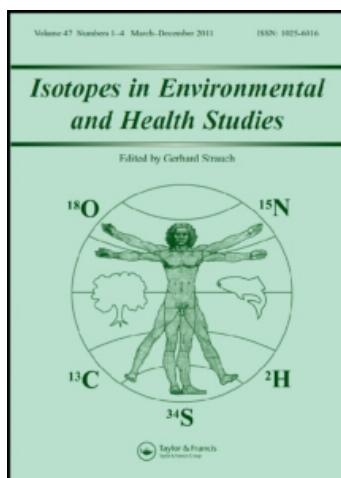
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Isotopes in Environmental and Health Studies

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Administering labelled water to exclusively breast-fed infants in studies involving stable isotope dilution techniques

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Adequacy of Milk Intake During Exclusive Breastfeeding: A Longitudinal Study

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Manuscripts to “Pediatrics for Parents” (600 – 1500 words)

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Can mothers manage exclusive breastfeeding for 6 months?

It is recommended that infants are exclusively breast-fed for the first 6 months of life. Exclusive breastfeeding is where the infant is given nothing else but breast milk – not even water. Breast milk can be given through breastfeeding or feeding expressed breast milk through for instance a bottle or a cup. According to the World Health Organization (WHO), exclusive breastfeeding for the first 6 months of life provides the optimal nutrition for a healthy infant.

Breastfeeding provides many benefits for both mother and infant. These benefits may be greater when breastfeeding is the exclusive nourishment given to the infant, and when exclusive breastfeeding is sustained for longer periods of time (up to 6 months). However, only relatively few mothers manage to exclusively breast-feed for 6 months, and this has only improved very little in spite of many initiatives to promote breastfeeding.

Many circumstances, such as medical complications, work commitments, lack of professional and/or family support, and lack of knowledge can make exclusive breastfeeding difficult to initiate and sustain. However, for those mothers who do successfully initiate exclusive breastfeeding, one of the main reasons for stopping exclusive breastfeeding before 6 months is the perception that they have an insufficient milk supply. Such mothers report statements like “not having enough milk”, “baby being too hungry”, or that they are “breastfeeding too often” or “breastfeeding for too long” as reasons for stopping exclusive breastfeeding. Some health professionals have the same view, and they may advise mothers to ‘top up’ with formula milk, or even to stop breastfeeding. Therefore, the First-Feed study was set out to explore whether normal mothers produce enough milk for their babies for 6 months during exclusive breast-feeding.

The actual milk intake of a breastfed baby is hard to measure precisely. The most commonly used method to measure milk intake is to instruct the mother to weigh the baby before and after each feed, while recording accurate time and duration of feeds. However, this method is intrusive on the breastfeeding routine and puts an extra burden on the mother, and it has probably underestimated the amount of milk the baby takes during exclusive breastfeeding in older studies. Instead, it is custom to measure infant growth (particularly weight) frequently, because normal infant growth compared to growth charts is a reasonably good indication that an infant is getting enough milk.

In a quest to find out if infants do get enough milk when exclusively breastfed for 6 months, the First-Feed study was set up with the aim of using an accurate but less intrusive method of measuring milk intake in infants that were exclusively breast-fed to 6 months of age (*Pediatrics* 2011; 128(4): e907-e914). The mothers were recruited from breastfeeding groups in Scotland, UK. They were all very motivated to breast-feed and were well-supported. The method included giving a non-radioactive isotope of water to the baby, and subsequently measure how quickly this water is eliminated in the urine. The quicker the water is eliminated, the more milk the infant has taken in. At the same time, the infants were measured frequently to ensure that they were growing normally.

The First-Feed study found that by 15 weeks of age babies had a very high intake of breast milk, higher than expected from older studies which had used weighing of the baby before and after a feed. Average breast milk intake by babies in the study was almost 34 ounces (1 liter) per day at 15 weeks of age, and their milk intake increased as they got older. This amount of milk intake was enough to cover their energy requirements and the infants were growing well. At the same time, the

average time per day and per feed spent on breastfeeding decreased with age, suggesting the babies got more efficient at taking the milk needed to cover their energy requirements. There was therefore no evidence that breastfeeding became more demanding on the mother over time – on the contrary, it seemed to become easier over time.

The biology of lactation has been thoroughly investigated, and it is well known how milk production is regulated. Previous studies have found that “supply follows demand”. Therefore, when the baby grows and the need for breast milk increases, there is a responsive increase in milk production by the mother. This regulation makes it possible for mothers to successfully breastfeed siblings or twins by producing very high volumes of breast milk. It is also possible for a lactating mother who is breastfeeding one infant to increase her milk production by increasing the degree of emptying of the breasts through expressing any extra milk at each feed for a few days. The First Feed study shows fairly conclusively that mothers manage exclusive breastfeeding quite easily if they are well supported. They manage by producing large amounts of milk for their babies, larger amounts than we had expected based on older studies, and by increasing the amount of milk provided as their babies get older. Based on the First-Feed study, the idea that mothers have insufficient milk is a myth.

But if there is no biological barrier to exclusive breastfeeding for 6 months, and the benefits of doing so are well documented, why is exclusive breastfeeding to 6 months so rare? This might be explained by the fact the breastfeeding is a behavioural activity, that is strongly influenced by environmental and social factors. Many studies have shown that mothers’ intention or determination to breastfeed predicts their success in doing so. In many parts of the world, the lowest breastfeeding rates are to be found among the most deprived populations. This suggests that breastfeeding does require resources from the mother; she needs to want to breastfeed, and she needs lots of appropriate support from both the general community, from health professionals, and from the closer social and family network around her. The evidence from the First-Feed study should encourage mothers, families, and health professionals to believe that if mothers are well-supported and determined to breastfeed exclusively to 6 months; this can be achieved without any biological barrier, simply by understanding that milk supply will follow the demand of the baby.

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